Acta Obstetricia et Gynecologica Scandinavica

Published by

The Scandinavian Association of Obstetricians and Gynaecologists

Editor

Professor Axel Ingelman-Sundberg

Address

Sabbatsbergs kvinnoklinik, 113 82 Stockholm, Sweden

Vol 52, 1973

Printed in Sweden by

Almqvist & Wiksell, Uppsala 1973

INDEX

Letters A and Ä—see under A, letters Ø and Ö—see under O

Ala-Kerola, L., Roentgen diagnosis of female stress incontinence, suppl. 23
Ambrose, A see Teoh, E. S., 323
Amedt, B see Svanberg, L., 141
Aubert, M L., see Persson, B., 63
Badr M., see Fayad, M. M 69
Bahna, S L see Bjerkedal, T 345
Bakke, T A new mechanical instrument for the measurement of fibroelasticity with special reference to its use in the assessment of the consistency of the uterine cervix, 277
Bartsch, F Fetale Erythrocyten im mütterlichen Blut und Immunprophylaxe der Rh-Immunisierung, suppl. 20
Beckman, L von Schoultz, B and Stigbrand, T Detection of the pregnancy zone protein by means of an immunodiffusion method, 157
Bergqvist, P Gastrointestinal tract reaction to radiation therapy in cancer of the cervix, measured by the vitamin A absorption test, 59
Bergqvist, P and Brodtkorb C Ultrasonic fetal cephalometry in pre-eclampsia, 249
Bjerkedal, T and Bahna, S L. The course and outcome of pregnancy in women with epilepsy 45
Brodtkorb C see Bergqvist, P 49
Brummer H C and Craft, I. L. Prostaglandin $F_{2\alpha}$ and labour 273
Carlström, K see Persson, B. 63
Carlström, K see Fursdydam, M 373
Cloeren, S E see Lippert, T H., 131
Craft, I L. see Brummer, H. C 273
Daremeno V A see Martinez, L. R., 391
Erkkola R Kanto, L and Pekkarinen, A The transfer of diazepam across the placenta during labour, 167
Fayad, M M Youssef, A F Zakrase, M Kamel, M. and Badr M The ureterocalyceal system in normal pregnancy 69
Fiber J P see Persson, B 63
Frank, M J see Tervila, L., 177
Frank, M J see Tervila, L. 309
Hoffmann, B see Ulstein, M 795
Forssman, L ^{133}Xenon clearance from the human non-pregnant uterus, 177
Fredholm, A M see Törnqvist, A., 25
Frede R R see Lippert, T H., 131
von Friesen, B The Aukerdikator in therapeutic abortion, 191
Fuchs, F see Lauersen, N H 77
Fursdydam, M and Carlström, K. Amenorrhoea following use of combined oral contraceptives, 373
Goecke, C see Tervila, L., 23
Gustavii, B Studies on the mode of action of intra-amniotically and extra-amniotically injected hypertonic saline in therapeutic abortion, suppl. 25
Gottorm, E., see Nicolaisen, H. H., 253
Gyrning, I see Svanberg, L., 141
Hackl, H Metabolism of glucose in the human endometrium with special reference to fertility and contraception, 135
Haram, K., see Segrn, N 133
Hashiba, N., see Nakano, R., 171
Hayashi, K., see Tojo, S 335
Henn, P and Thorén, C. Congenital arrhythmias with supraventricular tachycardia in the perinatal period, 381
Hunnekamp, M., see Lippert, T H., 131
Hjertén, S., see Johansson, L.-E., 345
Höglund, A., Jochmon, L. Ingelman-Sundberg, A. and Odeblad, E. Acridine orange in gynaecologic cancer. II. The effect of stain receptors on some proton magnetic resonance parameters, 1
Ingelman-Sundberg, A., see Höglund, A., 1
Ivy B J see Rooth, G 47
Jacobsen, B. Brock, see Nielsen, F Henning, 83
Järvinen, P A see Ylikorkala, O 229
Jensen, H The effect of paracervical block on dilatation and uterine activity 13
Jochmon, L., see Höglund, A 1
Johannsen, E. D B Plasma levels of progesterone and oestradiol after injections of 17α-hydroxyprogesterone caproate during the luteal phase of the normal menstrual cycle, 37
Johansen, P see Tornqvist, A 25
Johansson, L. E. The clinical value of amniotic fluid analysis in pregnancies complicated by Rh-isoimmunization or hepatosis, 113
Johansson, L.-E. and Hjertén, S Free zone electrophoresis of amniotic fluid in normal pregnancies and in pregnancies complicated by haemolytic disease 345
Jouppila, P see Ylöstalo, P 299
Kaiho H Cytogenetic and clinical study on 100 cases of primary amenorrhoea, suppl 4
Kamel, M see Fayad, M M 69
Kangas, L see Erkkola, R., 167
Katayama, K see Nakano, R 269
Kauppila, A see Ylöstalo, P 299
Kivalo, I see Tervila, L 177
Kivalo, I see Tervila, L., 309
Kivinen, S see Ylöstalo, P 299
Klingenberg, I Measurement of uterine blood flow in non-pregnant women by electromagnetic flowmeter 317
Kotsuga, F see Nakano, R., 171
Kotsuga, F see Nakano, R., 269
Kullander S and Sandahl, B. Fetal chromosome analysis after transcervical placental biopsies during early pregnancy 355
Larsen, M. Saltorp Treatment of ovarian carcinoma, see suppl. 22

Lau, Y K., see Teoh E. S., 323
Lauersen, N. H and Fuchs, F. Experience with Shirodkar's operation and postoperative alcohol treatment, 77
La rsen, B., Twin pregnancy 367
Lefèvre H see Mogensen, P 109
Levi, S. and Smets, P Intra-uterine fetal growth studied by ultrasonic biparietal measurements, 193
Lippert, T H Cloeren, S E., Fridrich, R. and Hinselmann, M Continous recording of uteroplacental blood volume in the human, 131
Liukko, P see Pulkkinen, M O 9
Lundvall, F Treosulfan in the treatment of ovarian carcinoma, see suppl.
Lunell, N-O., Persson, B. and Öbqvist, G The effects of an oral combined contraceptive on plasma levels of glucose, free fatty acids, glycerol, D-β hydroxybutyrate and triglycerides, 23
Lunell, N-O see Persson, B 63
Martinez L. R., Degennaro, V A. and Veloso, E. J A quiet type I hyperlipoproteinemia in pregnancy 391
McBride, R., see Rooth, G 47
Mizuno, T see Nakano R., 171
Mizuno, T see Nakano, R., 269
Mogensen, B. Invasive mole and gestational choriocarcinoma in Denmark, 1940–1969 211
Mogensen, P Rødbro, P and Lefèvre H Radioisotope renography in the surgical management of carcinoma of th cervix, 109
Molin, J see Ulmsten, U 147
Nakano R., Kotsuji, F Mizuno, T Hashiba, N Washio, M and T jo, S. Response to luteinizing hormone releasing factor (LRF) in normal subjects and anovulatory patients, 171
Nakano R., Mizuno, T Kotsuji, F Katayama, K Washio, M and T jo, S "Triggering of ovulation aft r infusion of synthetic luteinizing hormone releasing factor (LRF), 269
Nicolaisen, H. H., Pedersen, H., Guttorm, E. and Rebbe H. Post vulatory endometrial development in women with I U D 253
Nielsen, F Hasling, Jacobsen, B. Brock and Rolschau, J Pregnancy complicated by extreme hyperlipaemia nd foam-cell accumulation in placenta, 83
Nielsen, N. Chr Hidroadenoma of the vulva, 387
Nilsson, I M see Svanberg, L 141
Nordvall, S A device f r v ginal eedle aspiration biopsy 91
Nygren, K-G. and Persson, B. H Uterus didelphys with an bortion into unilateral imperf rate agina, 187
Odeblad, E see Hoglund, A., 1
Öbqvist, G see Lunell, N-O 23
Osawa, E see T jo, S., 335
Palmgren, B Wahlén, T and Wallander B Toxaemia and cigarette smoking during pregnancy 183
Pedersen, H see Nicolaisen, H. H 253
Pekkarinen, A., see Erkkola, R 167
Peralaminov L. S. The ffect of normal and abnormal labour on the foetus, 79
Persson, B see Lunell, N-O., 23
Persson, B., Lunell, N-O Aubert, M L., Carlström, K. and Felber J P Determination of plasma human chorionic somatomammotrophin and urinary oestriol in diabetic pregnancies, 63
Persson, B. H., see Nygren, K.-G 187
Piiroinen, O see Pulkkinen, M. O 9
Poulsen, E. Fangel. The induction and stimulation of labour with *buccal desaminooxytocin and oxytocin tablets,* 303
Pulkkinen, M O., Liukko P Piiroinen, O. and Rauramo, L. Induction of midtrimester abortion by increasing intra-uterine volume 9
Pulkkinen M O Reply to Dr Goodlin's Letter to the editor ol. 51 93
Punnonen, R. Effect of castration and peroral estrogen therapy on the skin, suppl. 1
Raines, T see Ylikorkala, O 229
Ratnam, S S see Teoh, E. S 323
Rauramo, L., see Pulkkinen, M O 9
Rebbe H see Nicolaisen, H H., 253
Rødbro P see Mogensen, P 109
Rolschau, J see Nielsen F Hasling, 83
Rooth, G McBride R. and Ivy B. J Fetal and maternal pH measurements, 47
Säfgren, N and Haram, K. Diazepam (Valium®) as an anaesthetic for operati e vaginal delivery 153
Said, S. A. H. and Wide L. Serum levels of FSH and LH f llowing normal parturition, 361
Samsioe, G see Tornqvist, A., 25
Sandahl, B se Kullander S., 355
Sandstrom, B Calf Blood flow during normal Pregnancy *199*
Sandstr m, B. and von Schmalensee G Intraarterial blood pressure and peripheral vascular resistance in the leg and arm during the f rst trimester of primipregnancy 289
on Schmalensee G see Sandstrom, B., 289
von Schoultz, B. and St gbrand, T Purification of the pregnancy zone protein, 51
on Schoultz, B see Beckman, L., 157
Sele V see Starup J 259
Sjostedt, E., see Ylikorkala, O 229
Smets, P see Levi, S 193
Soderberg, G see Westin, B 41
Sørensen, H Mygind Ovarian carcinoma treatment ith treosulfan, see suppl
Starup, J and Sele V Premat re ovarian failure 259
Stigbrand, T see on Schoultz, B., 51
Stigbrand, T see Beckman, L 157
S anberg, L., Astedt, B Gynning, I and Nilsson, I. M F bri degradation products during postoperative radiotherapy of ovarian carcinoma, 141
Teoh, E. S., Lau, Y K Ambrose A. nd Ratnam, S. S Amniotic fluid creatinine uric acid and urea as indices of gestational ge 323
Tervila, L., Vartiainen, E Kivalo, I Finnila, M J and V yrynen, M The ffect f oxygen ventil tion and asodilator on terine perfusion, fetal oxygen and acid-base b lance I A study in healthy gra idae 177
T rvil., L Goecke C and Timonen, S., Estimation f gestosis in pregnancy (EPH-gestosis), 235
Tervila, L., Vartiainen, E., Kivalo, I Finnilä, M J and V yrynen, M The effect of ygen entilatio and

vasodilator on uterine perfusion, fetal oxygen and acid-base balance. II A study in abnormal gravidae, 309
Thorén, C., see Herbs, P 381
Tiitinen, R see Ylikorkala, O., 229
Timen, J : Ovarian carcinoma—a 10-year series from provincial hospital, 103
Timonen, S see Tervilä, L., 235
Tojo, S see Nakano, R., 171
Tojo, S see Nakano, R 269
Tojo, S Hayashi, K. and Oshima, E. Stimulation of ovulation with F-6066 cyclofenil: bis(p-acetoxyphenyl)-cyclohexylidenemethane, 335
Törnqvist, A., Fredholm, A-M Jonsson, F and Samsioe, G On the passage of an exogenous d amino oxidase inhibitor to the amniotic fluid, 25
Ulmsten, U. and Malm, J Percutaneous nephropyelostomy in postrenal obstruction, 147
Ulstein, M Fertility of husbands at homologous insemination, 5
Ulstein, M Fertility of donors at heterologous insemination, 97
Ulstein, M Fertility motility and penetration in cervical mucus of freeze-preserved human spermatozoa, 205
Ulstein, M and Fjällbrant, B Interrelation of different parameters at cervical mucus penetration of spermatozoa, 291
Vaclavaková, V A method of measuring the inner uterine diameter by an ultrasonic technique, 161
Vartiainen, E., see Tervilä, L., 177
Vartiainen, E., see Tervilä, L., 309
Väyrynen, M., see Tervilä, L., 177
Väyrynen, M., see Tervilä, L., 309
Vehaskari, A., see Ylöstalo, P 299
Veloso P. J see Martinez, L R., 391
Vuopala, S see Ylöstalo, P 221
Wahlén, T see Palmgren, B 183
Wallander B see Palmgren, B., 183
Wasada, M., see Nakano, R., 171
Wasada, M see Nakano, R., 269
Westin, B. and Soderberg, G Equipment for direct FHR monitoring during labour, 41
Wide, L., see Saad, S. A. H 361
Ylikorkala, O., Sjöstedt, E Järvinen, P A., Tiitinen, R. and Ranta, T Trimethoprim-sulfonamide combination administered orally and intravaginally in the first trimester of pregnancy: its absorption into serum and transfer to amniotic fluid, 229
Ylöstalo, P and Vuopala, S Contraceptive treatment with low doses of gestagens in cases with medical history of hepatosis in pregnancy 221
Ylöstalo, P Kauppila, A Jouppila, P Kivinitty K. and Vehaskari, A Hepatic and adrenocortical function during cyclophosphamide show therapy 299
Youssef, A. F see Fayad, M. M., 69
Zahran, M., see Fayad, M M 69

Acta Obstet Gynec Scand 52: 1–4 1973

ACRIDINE ORANGE IN GYNECOLOGIC CANCER

II. The Effect of Stain Receptors on Some Proton Magnetic Resonance Parameters

Astrid Höglund, Ingemar Joelsson, Axel Ingelman-Sundberg and Erik Odeblad

From the Department of Medical Physics (Head: E. Odeblad, M.D., Ph.D.), Umeå Universitet Umeå and the Department of Obstetrics and Gynecology (Head: A. Ingelman-Sundberg, M.D.), Karolinska Institute, Sabbatsberg Hospital, Stockholm, Sweden

Abstract. The methyl line of the proton magnetic resonance spectrum of acridine orange zinc trichloride, dissolved in heavy water, has been studied. This line consists of two overlapping components with different widths. A mathematical technique has been developed to resolve the two components quantitatively. Their relative proportion depends on the acid-base state of the molecular environment. If the stain is adsorbed on albumin the narrow component quickly disappears, while adsorption on RNA gives rise to a broadening of the narrow line. With large amounts of albumin or RNA, both components disappear. DNA strongly adsorbs acridine orange with complete disappearance of all NMR spectral lines. Mitochondria, submitochondrial particles and microsomes cause some broadening of the narrow component of the methyl line. Due to the obvious alterations of the NMR spectrum, in the circumstances mentioned, this type of spectroscopy may be used for the biophysical characterization of interaction mechanisms between acridine orange and various proteins, nucleic acids and cell fractions.

In the first paper of this series (2) the NMR spectrum of acridine orange zinc chloride was studied and assignments of spectral lines were performed. The methyl line had a high intensity and was shown to consist of two components with nearly the same chemical shift (Fig. 1). The methyl resonances were therefore selected for special studies on the interaction of acridine orange with stain receptors. The lines of the ring bound protons were also observed to some extent. The stain receptors studied in this paper were albumin, RNA, DNA and a few cell fractions.

THEORETICAL

A special analysis of the components of the methyl line had to be performed. Each component of the complex NMR line is assumed to follow a Lorentz function as NMR lines should do when liquid specimens are examined (Bloch, 1946)

$$y = \frac{1}{1+(x/h)^2} \quad (1)$$

Here y = signal amplitude = X''/X''_{max} where X'' stands for RF absorption susceptibility and x is distance from peak centre in Hz. Thus, at $x = 0$, $y = 1$. The line half-width is denoted h.

As there are two components, we index the narrow one n and the broad one b and each is assumed to contribute the fractions p_n and p_b to the line amplitude (Fig. 2)

$$y = p_n \frac{1}{1+(x/h_n)^2} + p_b \frac{1}{1+(x/h_b)^2} \quad (2)$$

Here h_n and h_b are the line half widths of the narrow and broad components. With the purpose of determining the four parameters p_n, p_b, h_n and h_b the following considerations are made:

A. For large values of x (i.e. far away from the peak centre) because the contribution of the narrow component is negligible, we find approximately

$$y = p_b \frac{1}{(x/h_b)^2} \quad (3)$$

which gives

$$p_b h_b^2 = yx^2 \quad (4)$$

Here yx^2 is (1) evaluated for various values of x on averaged NMR recordings and (2) extrapolated to high values of x. The extrapolated value is taken as the accepted value for $p_b h_b^2$.

1 731941

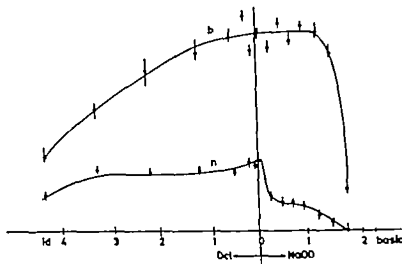


Fig. 3. Integrated intensities (relative values) of the narrow ( ) and broad (*b*) components at various molar proportions of NaOD and acridine orange and DCl and acridine orange respectively.

MATERIALS

The materials used are as follows:

D_2O and acridine orange zinc chloride, the same as in Part I in this series.

Albumin, human serum albumin for laboratory work, manufactured by Kabi Co., Stockholm, No. 1067. Serial No. R4 23.

RNA. Yeast nucleic acid manufactured by Sigma Chemical Co. (from Torula yeast, grade VI, lot No. 11 6-B 2520.

DNA. Calf thymus nucleic acid sodium salt, type V manufactured by Sigma Chemical Co., lot No. 53 B 0200. The following cell fractions were used:

(*a*) *Rat liver mitochondria*. Sorvall SS-34 centrifuge, 13 000 rpm for 15 min, g 8 000, 60 mg/ml of proteins.

(*b*) *Rat liver microsomes*. Spencer centrifuge, rotor 40, 40 000 rpm for 60 min, g 105 000. Wet liver contents 0.5–1.0 g/ml.

(*c*) *Beef heart mitochondria*. Sorvall SS-34 centrifuge, 12 000 rpm for 10 minutes, g 17 000. 55.8 mg/ml of proteins.

(*d*) *Beef Heart MASP* (submitochondrial particles). Spencer centrifuge, rotor 40, 40 000 rpm for 40 min, g 105 000. 30 mg/ml of proteins.

RESULTS

A. *Effect of acid and alkaline environment*

The signal parameters were studied upon addition of various amount of NaOD and DCl. The ring proton signals showed some alterations, but the changes in the methyl components were more striking as shown in Fig. 3. Following addition of NaOD the ring proton signals were broadened, reduced in amplitude, and their structure was altered. The narrow methyl component declined rapidly in amplitude to about 30% of its original height at equal molar amounts of NaOD and acridine orange. At still larger amounts of NaOD both the narrow and the broad components disappeared. Reduced solubility of acridine orange may have contributed to this result. When DCl was added, some alterations also occurred in the ring proton signals, but also here the most obvious changes occurred in the methyl lines. There was a slow gradual reduction of the broad component. Even after the addition of large amounts of DCl, the narrow component persisted to some extent, while the broad component had completely disappeared.

B. *Effects of the presence of some biochemical compounds*

Addition of *albumin* caused a gradual disappearance of the narrow methyl component. It disappeared completely after the addition of albumin up to a weight fraction of 0.6 of that of acridine orange (Fig. 4). The addition of larger amounts of albumin finally caused the broad methyl peak to disappear completely as well.

Addition of *RNA* caused broadening of the narrow component of the methyl line. In equal weight proportions of RNA and acridine orange the width amounted to about twice the original. At considerably larger amounts of RNA, both methyl components became strongly broadened and disappeared.

Addition of *DNA* was accompanied by very

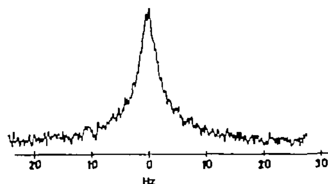


Fig 1 The methyl line of acridine orange. Scale is given in Hz (x-coordinate)

B For very small values of x i.e. in the immediate vicinity of the peak centre, the variation of y as a function of x is almost entirely dependent on the narrow component. We find approximately

$$1-y = p_n\left(\frac{x}{h_n}\right)^2 \tag{5}$$

which gives

$$\frac{p_n}{h_n^2} = \frac{1-y}{x^2} \tag{6}$$

Here $1-y$ and x^2 are evaluated from the experimental recordings and the quotient obtained is extrapolated to $x=0$. This extrapolated value is taken as the value of the quotient p_n/h_n^2

C. For moderately small values of x within the upper curvature of the signal we find approximately

$$1-y = p_n\left(\frac{x}{h_n}\right)^2 + p_b\left(\frac{x}{h_b}\right)^2 \tag{7}$$

By derivation of this expression we find

$$-\frac{(dy/dx)}{2x} = \frac{p_n}{h_n^2}+\frac{p_b}{h_b^2} \tag{8}$$

The value of $-(dy/dx)/2x$ is taken from the NMR recording, its average is calculated from 4 to 5 measurements and is regarded as the acceptable value of $p_n/h_n^2 + p_b/h_b^2$

D Finally by definition $p_n + p_b = 1$ By combinations of the equations (4) (6) (8) and (9) we can solve the four parameters p_n p_b, h_n and h_b.

The total number of protons under the signal is obtained by integration of the signal with the aid of the electronic integrator of the spectrometer

According to general theory

$$\text{Proton number} = \text{const}\int_{-\infty}^{\infty} y\,dx$$

$$= p_n\int_{-\infty}^{\infty}\frac{dx}{1+(x/h_n)^2} + p_b\int_{-\infty}^{\infty}\frac{dx}{1+(x/h_b)^2}$$

$$= \text{const.}\ \pi(p_n h_n + p_b h_b)$$

Thus, the relative contribution of each component to the total proton number equals maximum height times line width. After calibration with a known amount of protons in a signal, the constant can be evaluated and the proton number in each component determined.

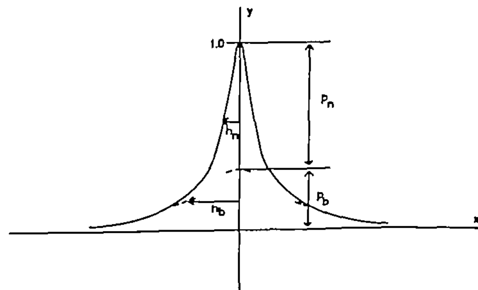


Fig 2 Schematic drawing of the methyl line and its resolution into two components, *n* and *b*

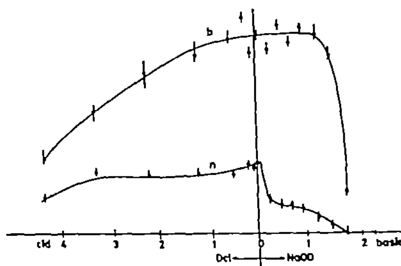


Fig. 3. Integrated intensities (relative values) of the narrow (*a*) and broad (*b*) components at various molar proportions of NaOD and acridine orange and DCl and acridine orange respectively.

MATERIALS

The materials used are as follows:

D_2O and acridine orange zinc chloride: the same as in Part I of this series.

Albumin, human serum albumin for laboratory work, manufactured by Kabi Co., Stockholm, No. 1067, Serial No. Rd 23.

RNA. Yeast nucleic acid manufactured by Sigma Chemical Co. from Torula yeast, grade VI, lot No. 11 4-B 2520.

DNA. Calf thymus nucleic acid sodium salt, type V manufactured by Sigma Chemical Co., lot No. 53 B 0200. The following cell fractions were used:

(*a*) *Rat liver mitochondria*. Sorvall SS-34 centrifuge, 13 000 rpm for 15 min, g 8 000, 60 mg/ml of proteins.

(*b*) *Rat liver microsomes*. Spencor centrifuge, rotor 40, 40 000 rpm for 60 min, g 105 000. Wet liver contents 0.1 g/ml.

(*c*) *Beef heart mitochondria*. Sorvall SS-34 centrifuge, 12 000 rpm for 10 minutes, g 17 000, 55.8 mg/ml of proteins.

(*d*) *Beef Heart MASP* (submitochondrial particles). Spencor centrifuge, rotor 40, 40 000 rpm for 40 min, g 105 000, 30 mg/ml of proteins.

RESULTS

A. *Effect of acid and alkaline environment*

The signal parameters were studied upon addition of known amounts of NaOD and DCl. The ring proton signals showed some alterations, but the changes in the methyl components were more striking as shown in Fig. 3. Following addition of NaOD the ring proton signals were broadened, reduced in amplitude and their structure was altered. The narrow methyl component declined rapidly in amplitude to about 30% of its original height at equal molar amounts of NaOD and acridine orange. At still larger amounts of NaOD both the narrow and the broad components disappeared. Reduced solubility of acridine orange may have contributed to this result. When DCl was added some alterations also occurred in the ring proton signals, but also here the most obvious changes occurred in the methyl lines. There was a slow gradual reduction of the broad component. Even after the addition of large amounts of DCl, the narrow component persisted to some extent, while the broad component had completely disappeared.

B. *Effects of the presence of some biochemical compounds*

Addition of *albumin* caused a gradual disappearance of the narrow methyl component. It disappeared completely after the addition of albumin up to a weight fraction of 0.6 of that of acridine orange (Fig. 4). The addition of larger amounts of albumin finally caused the broad methyl peak to disappear completely as well.

Addition of *RNA* caused broadening of the narrow component of the methyl line. I equal weight proportions of RNA and acridine orange the width amounted to about twice the original. At considerably larger amounts of RNA, both methyl components became strongly broadened and disappeared.

Addition of *DNA* was accompanied by very

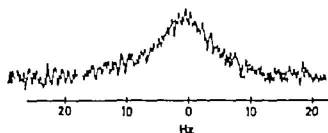


Fig 4 The broad component of the methyl line, after addition of 60% w/w of albumin.

strong adsorption of the stain. At equal amounts of DNA and acridine orange no methyl or ring proton signals were detectable in the spectrum (Fig. 5) The supernatant aqueous phase surrounding the strongly stained DNA gel showed only a few per cent of its original methyl peak activity indicating the strong affinity of the DNA for acridine orange.

C. *Effects of the presence of cell fractions*

All of the cell fractions were investigated in volumes of 0.1 and 0 6 ml per ml of a 2.5% acridine orange solution A slight broadening of the narrow component occurred in all samples. For the microsome fraction there also occurred a widening of the broad methyl component. No obvious alterations were seen in the ring proton signals.

DISCUSSION

Of the compounds investigated DNA seems to be especially active in accreting acridine orange. The apparent disappearance of the NMR lines is due to immobilization of the stain molecules upon binding to macromolecules. This reduced mobility enhances the magnetic relaxation of proton spins, leading to line broadening. The lines finally become so broad that they cannot be detected by the NMR technique used hence their disappearance" This locking of stain molecules to a macrocolecule may affect various parts of the stain molecule in different ways, thus leading to different degrees of broadening of various NMR components. This was evident when the binding action of the cell fractions was examined.

The role of the zinc atom in the binding of acridine orange is not clear and the present investigation does not give information pertinent to this problem. As is evident from Part I of this series, a number of other properties of the acridine orange molecule may also be of importance for its interaction with macromolecular systems.

Apparently NMR spectroscopy can be a tool to study both the mechanism and the degree of accretion between acridine orange and stain receptors. In view of the dependence of the methyl line structure on acid-base-state it is important to know the acid-base state of the system studied. Local acidic or alkaline environments on macromolecular surfaces may be as important as the general hydrogen-ion concentration. A number of biochemical systems have to be investigated with regard to their effect on the NMR spectrum of acridine orange before the binding mechanisms in vivo and the background to clinical applications can be sufficiently understood.

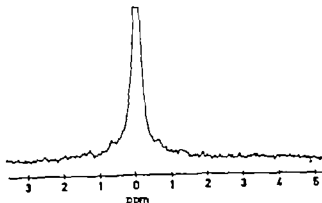


Fig 5 Spectrum of DNA with an adsorbed equal amount of acridine orange N methyl peak is detectable

ACKNOWLEDGEMENTS

We are indebted to Professor Lars Ernster Stockholms Universitet, for the cell fractions used and regarding Parts I and II in this series to Miss MaiLis Schlstedt for secretarial assistance and to Miss Astrid Odeblad for drawing the figures.

REFERENCES

1 Bloch, F Physical Rev 70 460 1946.
2 Höglund, A., Joelsson, I., Ingelman-Sundberg, A. & Odeblad, E. Acta Obstet Gynec 51 309 1972.

Submitted for publication J n. 1 197

Ingemar Joelsson
Department of Obstetrics and Gynecology
Sabbatsberg Hospital
113 8? Stockholm
Sweden

Acta Obstet Gynec Scand 52. 5–8 1973

FERTILITY OF HUSBANDS AT HOMOLOGOUS INSEMINATION

Magnar Ulstein

From the Department of Obstetrics and Gynecology Östra Sjukhuset (Head, Professor P Bergman), Göteborg S eden

Abstract. The seminal properties and female fertility factors were studied in 45 couples where homologous inseminations were performed. For 10 couples (group I) normal coitus was impossible. In this group there were 8 normal pregnancies and 1 miscarriage. For 35 couples (group II) the postcoital test showed persistently reduced penetration because of poor semen quality or hostility of the cervical mucus. In this group there ere 10 conceptions, 4 normal pregnancies and 6 miscarriages. The semen quality was more important prognostic than pathological findings in the women. In vitro sperm penetration of cervical mucus seemed to be the best criterion for judging the prognosis. Homologous insemination can be of value in the treatment of infertility but careful selection of patients is needed

Homologous insemination has two main indications. (I) I couples where coitus is impossible for anatomical, neurological or mental reasons (hypospadias, epispadias, vaginal deformities, retrograde ejaculation, impotence premature ejaculation, severe dyspareunia or vaginismus) (II). In couples with sterility of long duration where the postcoital test persistently shows poor penetration This may be caused by poor semen quality or cervical factors, or both.

Poor sperm penetration is accepted as a cause of infertility but the influence of semen quality on the penetration is not fully understood. By comparing the results of insemination in a group of couples with poor sperm penetration with a group with apparently normal sperm penetration, the influence of penetration can be demonstrated.

The aim of this investigation was to analyse the results of homologous insemination with special regard to the semen quality the postcoital test, and the in vitro sperm penetration of cervical mucus.

MATERIAL

The series consisted of 45 couples with indications for homologous insemination. Ten belonged to group I, in which normal coitus was impossible, and 35 to group II, in which the postcoital test showed poor sperm penetration. The ages of the women at the start of the homologous inseminations varied from 24 to 38 years of age. The duration of sterility varied from 2 to 9 years.

All of the women had normal anatomy of the fallopian tubes. For those patients in group II no convincing cause of infertility other than poor penetration was found. Totally 263 inseminations ere performed.

METHODS

Examination of the women was extensive, depending on the cause of infertility For most of the women insufflations, hysterosalpingography curettage, and for some *laparoscopy or laparotomy ere performed* The omen were instructed to keep basal body temperature chart over period of at least 3 months.

Semen analysis as performed according to the routine of the laboratory The semen samples were taken by masturbation after at least 3 days of continence and brought to the laboratory in plastic condom, specially made for this use The analyses and sperm penetration studies ere started within 2 hours after ejaculation. Volume, density percentage of living spermatozoa, percentage of motile spermatozoa, motility degree (graded from 0 to 4), percentage of abnormal spermatozoa, content of fructose and acid phosphatases in seminal plasma ere specified The following values for the laboratory findings ere considered pathological. volume 2 ml, density 60 millions ml, living 70%, motile 50% motility degree < 2, abnormal spermatozoa 50%, fructose 100 mg%, and acid phosphatases 20 000 IE/ml.

Examination of the men ith pathological semen properties as performed by urologist and treatment instituted according to the clinical findings (varicocele, prostatitis etc) The semen quality given in the tables as the mean for several samples from each man obtained during the period the inseminations ere performed.

Serum samples from the men were examined for sperm antibodies by the method of Kibrick, Belding & Merril (.)

I *vitro sperm penetration tests* were performed according to the method of Kremer (6) slightly modified (11) Cervical mucus with ovulatory characteristics, carefully pretested, as well as the test medium, Capillary tubes with internal diameter of 0.6 mm and length 41 mm

Table I *Results of homologous insemination*

	Group I	Group II
Patients	10	35
Conceptions	9	10
Live babies	8	4
Spontaneous abortions	1	6

were filled with cervical mucus and one end immersed in a reservoir containing semen. Incubation temperature was 37°C and penetration extent in mm was read at 3 hours.

The results were grouped as follows. Greatly reduced = th. foremost spermatozoa had penetrated less than 6 mm. Reduced = the foremost spermatozoa were in the range 6–19 mm from the immersed end of the capillary tube. Normal = the foremost spermatozoa were found 20 mm or more from the immersed end of the capillary tube

The postcoital test was performed at the expected time of ovulation and 2 5 hours after coitus. For each couple 2 or more tests were performed. Vaginal secretion taken from the posterior fornix, cervical secretion taken with forceps f rst from the lower part, and then from the upper part of the cervical canal, and secretion from the uterine cavity aspirated with a cannula were examined for the presence of spermatozoa. The extent of sperm penetration was classified as follows: Greatly reduced = in addition to the vaginal secretion, spermatozoa were found only in the secretion from the lower part of the cervical canal. Reduced = spermatozoa were observed in the secretion from the lower and the upper part of the cervical canal Normal = spermatozoa were found n the secretion from the upper part of the cervical canal and from the uterine cavity

Technic of insemination was chosen with regard to the indications. Only fresh ejaculates, never more than one hour fter jaculation, were used. Most of the ejaculates were taken by masturbation, but for one man having retrograde ejaculation special technics were required.

Estimation of ovulation time was made with the aid of temperature charts, cycle length, and xamination f the cervix and the cervical mucus.

Table II *Interrelation between male and female fertility status*

M le status	Female status: Normal Conceptions/ Couples	Pathological Conceptions/ Couples	Total Conceptions/ Couples
Normal semen qual ty	7/7	5/7	12/14
Poor semen quality or sperm antibodies serum	5/12	2/19	7/31
Total	12/19	7/?6	19/45

For group I patients cervical insemination combined with application in a plastic cap was used, hereby 0.5 ml of the ejaculate was deposited in the cervical canal with a plastic tube and the rest into a plastic cap covering the portio.

For group II patients in addition to cervical insemination and application in a plastic cap intrauterine insemination was used also The plastic tube was inserted just beyond the internal os and 0 1 ml of the ejaculate was injected, 0.5 ml was deposited in the cervical canal, and the rest in the plastic cap Only one inseminatuon was performed in each cycle.

The patients were allowed to lie in the lithotomy position for one hour after the insemination was performed. The cervical cap was removed after 1–8 hours.

Statistical calculations for comparing the differences between distributions utilized the chi square test. A dif ference was considered significant when $p < 0\ 05$

RESULTS

Of the 10 women in group I 9 conceived, and in group II 10 of 35 women conceived (Table I). One woman in group I and 6 in group II aborted spontaneously The other pregnancies went to term and all babies were healthy The woman in group I who did not conceive had cervicitis, and *her husband had oligospermia. There were signifi*cantly more conceptions in group I than in group II

There were normal findings in 8 of the women in group I and 11 in group II of these all in group I and 4 in group II conceived. In 24 of the women in group II there were some pathological findings such as cervicitis, erosion uterine hypoplasia, and small myomata Six of these women conceived.

In group I there were two and in group II there were 29 men with poor semen quality Conception occurred for one of the 2 cases in group I and for 6 cases in group II Four men in group II had in addition to poor semen quality sperm antibodies in their serum and for 2 of these cases conception was obtained (titres. 1/16 and 1/128) For the cases in which no conception was obtained the titres were 1/256 and 1/16384

Table II shows the combined influences of male and female fertility status on prognosis. The highest frequency of conception was found for couples without pathological findings. Of 12 women with normal findings but having husbands with poor semen quality only 5 conceived. Seven women with normal husbands had some pathological findings of these 5 conceived. In 19 couples there were both poor semen quality and

pathological findings in the women and only 2 of these women conceived.

Table III gives more detailed information about the influence of semen qualities in relation to conception. For sperm density percentage of motile spermatozoa, motility degree and content of acid phosphatases in seminal plasma the differences in conception rates were statistically significant. For volume, percentage of abnormal spermatozoa, and content of fructose in seminal plasma the differences were not significant.

The conceptions distributed according to in vitro sperm penetration are shown in Table IV There are statistically significantly more conceptions when normal penetration is present In 10 cases with greatly reduced in vitro sperm penetration no conception occurred. In 19 cases in vitro sperm penetration was normal, amongst these 17 conceptions occurred. In cases in which reduced in vitro sperm penetration was demonstrated 2 of 16 women conceived.

Postcoital tests could not be performed for cases in group I. The relationships between postcoital tests in vitro tests and conception for group II couples are detailed in Table V There are no statistically significant differences. In 21 cases the postcoital test showed a greatly reduced in vivo sperm penetration. Among these only 2 women

Table III. *Semen properties in relation to conception*

Semen property	Conception	No conception
Volume (ml)		
2	4	4
2	15	22
Density (millions/ml)		
40	5	15
40	14	11
Motile ( )		
50	5	18
50	14	8
Motility degree		
1 or 2	7	19*
3 or 4	12	7
Abnormal spermatozoa ( )		
30	6	14
30	13	12
Fructose (mg %)		
100	1	3
100	18	23
Acid phosphatase (I.U.)		
20 000	2	10*
20 000	17	16

* Significant at the 5 % level

Table IV *In vitro sperm penetration degree related to incidence of conception in the entire series*

Sperm penetration	Conception	No conception	Total
Greatly reduced	0	10	10
Reduced	2	14	16
Normal	17	2	19
Total	19	26	45

Table V *Results of in vitro sperm penetration test related to those of postcoital test in group II*

Number of conceptions in brackets

In vitro sperm penetration	Postcoital sperm penetration		
	Greatly reduced	Reduced	Total
Greatly reduced	10(0)	0	10(0)
Reduced	9(0)	6(2)	15(2)
Normal	2(2)	8(6)	10(8)
Total	21(2)	14(8)	35(10)

conceived, and in both cases the in vitro sperm penetration was normal In 14 cases the postcoital test showed reduced in vivo sperm penetration, of these 8 conceived, two having reduced and 6 normal in vitro sperm penetration

The average number of inseminations performed for the women who conceived was 3.4 Cases in which there were sperm antibodies in the serum required the highest number of inseminations to achieve conception, and cases with normal seminal findings required the lowest number The mean number of inseminations to reach conception was lower in women with normal findings than women with some pathological findings.

No complications of a serious nature were noted A few women had uterine cramps in connection with intra uterine inseminations.

DISCUSSION

The indications for homologous insemination in cases where normal coitus cannot take place are obvious Not so clear are the indications in couples who have normal intercourse. In the present series strict criteria for selecting the patients were kept. For group II a reduced or greatly reduced sperm penetration was found in repeated postcoital tests. In these cases intra-

Table I *Results of homologous insemination*

	Group I	Group II
Patients	10	35
Conceptions	9	10
Live babies	8	4
Spontaneous abortions	1	6

were filled with cervical mucus and one end immersed in a reservoir containing semen. Incubation temperature was 37°C and penetration extent in mm was read at 3 hours.

The results were grouped as follows. Greatly reduced—the foremost spermatozoa had penetrated less than 6 mm. Reduced—the foremost spermatozoa were in the range 6–19 mm from the immersed end of the capillary tube. Normal—the foremost spermatozoa were found 20 mm or more from the immersed end of the capillary tube.

The postcoital test was performed at the expected time of ovulation and 5 hours after coitus. For each couple or more tests were performed. Vaginal secretion taken from the posterior fornix, cervical secretion taken with forceps first from the lower part, and then from the upper part of the cervical canal, and secretion from the uterine cavity aspirated with a cannula were examined for the presence of spermatozoa. The extent of sperm penetration was classified as follows. Greatly reduced—in addition to the vaginal secretion, spermatozoa were found only in the secretion from the lower part of the cervical canal. Reduced—spermatozoa were observed in the secretion from the lower and the upper part of the cervical canal. Normal—spermatozoa were found in the secretion from the upper part of the cervical canal and from the uterine cavity.

Technic of insemination was chosen with regard to the indications. Only fresh ejaculates, never more than one hour after ejaculation, were used. Most of the ejaculates were taken by masturbation, but for one man having retrograde ejaculation special technics were required.

Estimation of ovulation time was made with the aid of temperature charts, cycle length, and examination of the cervix and the cervical mucus.

Table II *Interrelation between male and female fertility status*

	Female status		
Male status	Normal Conceptions/Couples	Pathological Conceptions/Couples	Total Conceptions/Couples
Normal semen quality	7/7	5/7	12/14
Poor semen quality or sperm antibodies in serum	5/12	2/19	7/31
Total	12/19	7/26	19/45

For group I patients cervical insemination combined with application in a plastic cap was used, whereby 0.5 ml of the ejaculate was deposited in the cervical canal with a plastic tube and the rest into a plastic cap covering the portio.

For group II patients in addition to cervical insemination and application in a plastic cap intrauterine insemination was used also. The plastic tube was inserted just beyond the internal os and 0.1 ml of the ejaculate was injected, 0.5 ml was deposited in the cervical canal, and the rest in the plastic cap. Only one insemination was performed in each cycle.

The patients were allowed to lie in the lithotomy position for one hour after the insemination was performed. The cervical cap was removed after 1–8 hours.

Statistic l calculations for comparing the differences between distributions utilized the chi square test. A difference was considered significant when $p < 0.05$.

RESULTS

Of the 10 women in group I 9 conceived, and in group II 10 of 35 women conceived (Table I). One woman in group I and 6 in group II aborted spontaneously. The other pregnancies went to term and all babies were healthy. The woman in group I who did not conceive had cervicitis, and her husband had oligospermia. There were significantly more conceptions in group I than in group II.

There were normal findings in 8 of the women in group I and 11 in group II, of these all in group I and 4 in group II conceived. In 24 of the women in group II there were some pathological findings such as cervicitis, erosion, uterine hypoplasia, and small myomata. Six of these women conceived.

In group I there were two and in group II there were 29 men with poor semen quality. Conception occurred for one of the 2 cases in group I and for 6 cases in group II. Four men in group II had in addition to poor semen quality sperm antibodies in their serum and for 2 of these cases conception was obtained (titres 1/16 and 1/128). For the cases in which no conception was obtained the titres were 1/256 and 1/16384.

Table II shows the combined influences of male and female fertility status on prognosis. The highest frequency of conception was found for couples without pathological findings. Of 12 women with normal findings but having husbands with poor semen quality only 5 conceived. Seven women with normal husbands had some pathological findings, of these 5 conceived. In 19 couples there were both poor semen quality and

pathological findings in the women and only 2 of these women conceived.

Table III gives more detailed information about the influence of semen qualities in relation to conception. For sperm density, percentage of motile spermatozoa, motility degree and content of acid phosphatases in seminal plasma the differences in conception rates were statistically significant. For volume, percentage of abnormal spermatozoa, and content of fructose in seminal plasma the differences were not significant.

The conceptions distributed according to in vitro sperm penetration are shown in Table IV. There are statistically significantly more conceptions when normal penetration is present. In 10 cases with greatly reduced in vitro sperm penetration no conception occurred. In 19 cases in vitro sperm penetration was normal, amongst these 17 conceptions occurred. In cases in which reduced in vitro sperm penetration was demonstrated 2 of 16 women conceived.

Postcoital tests could not be performed for cases in group I. The relationships between postcoital tests in vitro tests and conception for group II couples are detailed in Table V. There are no statistically significant differences. In 21 cases the postcoital test showed a greatly reduced in vivo sperm penetration. Among these only 2 women conceived, and in both cases the in vitro sperm penetration was normal. In 14 cases the postcoital test showed reduced in vivo sperm penetration, of these 8 conceived, two having reduced and 6 normal in vitro sperm penetration.

The average number of inseminations performed for the women who conceived was 3.4. Cases in which there were sperm antibodies in the serum required the highest number of inseminations to achieve conception, and cases with normal seminal findings required the lowest number. The mean number of inseminations to reach conception was lower in women with normal findings than women with some pathological findings.

No complications of a serious nature were noted. A few women had uterine cramps in connection with intrauterine inseminations.

Table III. *Semen properties in relation to conception*

Semen property	Conception	No conception
Volume (ml)		
2	4	4
> 2	15	22
Density (millions/ml)		
60	5	15
60	14	11
Motile ( )		
50	5	18
50	14	8
Motility degree		
1 or 2	7	19
3 or 4	12	7
Abnormal spermatozoa ( )		
50	6	14
50	13	12
Fructose mg ...)		
100	1	3
100	18	23
Acid phosphatase (I.E.)		
20 000	2	10*
20 000	17	16

Significant at the 5% level.

Table IV. *In vitro sperm penetration degree related to incidence of conception in the entire series*

Sperm penetration	Conception	No conception	Total
Greatly reduced	0	10	10
Reduced	2	14	16
Normal	17	2	19
Total	19	26	45

Table V. *Results of in vitro sperm penetration test related to those of postcoital test in group II*

Number of conceptions in brackets

In vitro sperm penetration	Postcoital sperm penetration: Greatly reduced	Reduced	Total
Greatly reduced	10(0)	0	10(0)
Reduced	9(0)	6(2)	15(2)
Normal	2(2)	8(6)	10(9)
Total	21(2)	14(8)	35(10)

DISCUSSION

The indications for homologous insemination in cases where normal coitus cannot take place are obvious. Not so clear are the indications in couples who have normal intercourse. In the present series strict criteria for selecting the patients were kept. For group II reduced or greatly reduced sperm penetration was found in repeated postcoital tests. In these cases intra-

Table I *Results of homologous insemination*

	Group I	Group II
Patients	10	35
Conceptions	9	10
Live babies	8	4
Spontaneous abortions	1	6

were filled with cervical mucus and one end immersed in a reservoir containing semen. Incubation temperature was 37°C and penetration extent in mm was read at 3 hours.

The results were grouped as follows. Greatly reduced = the foremost spermatozoa had penetrated less than 6 mm. Reduced = the foremost spermatozoa were in the range 6–19 mm from the immersed end of the capillary tube Normal = the foremost spermatozoa were found 20 mm or more from the immersed end of the capillary tube

The postcoital test was performed at the expected time of ovulation and 2–5 hours after coitus. For each couple [illegible] or more tests were performed. Vaginal secretion taken from the posterior fornix, cervical secretion taken with forceps first from the lower part, and then from the upper part of the cervical canal, and secretion from the uterine cavity aspirated with a cannula were examined for the presence of spermatozoa. The extent of sperm penetration was classified as follows. Greatly reduced = in addition to the vaginal secretion, spermatozoa were found only in the secretion from the lower part of the cervical canal. Reduced = spermatozoa were observed in the secretion from the lower and the upper part of the cervical canal. Normal = spermatozoa were found in the secretion from the upper part of the cervical canal and from the uterine cavity

Technic of insemination was chosen with regard to the indications. Only fresh ejaculates, never more than one hour after ejaculation, were used. Most of the ejaculates were taken by masturbation, but for one man having retrograde ejaculation special technics were required.

Estimation of ovulation time was made with the aid of temperature charts, cycle length, and examination of the cervix and the cervical mucus.

Table II *Interrelation between male and female fertility status*

	Female status		
Male status	Normal Conceptions/ Couples	Pathological Conceptions/ Couples	Total Conceptions/ Couples
Normal semen quality	7/7	5/7	12/14
Poor semen quality or sperm antibodies in serum	5/12	[illegible]/19	7/31
Total	1[illegible]/19	7/26	19/45

For group I patients cervical insemination combined with application in a plastic cap was used, hereby 0.5 ml of the ejaculate was deposited in the cervical canal with a plastic tube and the rest into a plastic cap covering the portio.

For group II patients in addition to cervical insemination and application in a plastic cap intrauterine insemination was used also. The plastic tube was inserted just beyond the internal os and 0.1 ml of the ejaculate was injected, 0.5 ml was deposited in the cervical canal, and the rest in the plastic cap. Only one insemination was performed in each cycle

The patients were allowed to lie in the hibotomy position for one hour after the insemination was performed. The cervical cap was removed after 1–8 hours.

Statistical calculations for comparing the differences between distributions utilized the chi square test. A difference was considered significant when $p < 0.05$

RESULTS

Of the 10 women in group I 9 conceived, and in group II 10 of 35 women conceived (Table I). One woman in group I and 6 in group II aborted spontaneously The other pregnancies went to term and all babies were healthy The woman in group I who did not conceive had cervicitis, and her husband had oligospermia. There were significantly more conceptions in group I than in group II

There were normal findings in 8 of the women in group I and 11 in group II of these all in group I and 4 in group II conceived In 4 of the women in group II there were some pathological findings such as cervicitis, erosion uterine hypoplasia, and small myomata. Six of these women conceived.

In group I there were two and in group II there were 29 men with poor semen quality Conception occurred for one of the [illegible] cases in group I and for 6 cases in group II Four men in group II had in addition to poor semen quality sperm antibodies in their serum and for [illegible] of these cases conception was obtained (titres. 1 16 and 1/128). For the cases in which no conception was obtained the titres were 1 [illegible]56 and 1 16384

Table II shows the combined influences of male and female fertility status on prognosis. The highest frequency of conception was found for couples without pathological findings Of 1[illegible] women with normal findings but having husbands with poor semen quality only 5 conceived. Seven women with normal husbands had some pathological findings of these 5 conceived In 19 couples there were both poor semen quality and

Acta Obstet Gynec Scand 52: 9–12, 1973

INDUCTION OF MIDTRIMESTER ABORTION BY INCREASING INTRA UTERINE VOLUME

M. O. Pulkkinen, P. Liukko, O. Piiroinen and L. Rauramo

From the Department of Obstetrics and Gynecology (Head: Professor L. Rauramo), University of Turku, Turku, Finland

Abstract. The intra-uterine volume of 10 obstetrically normal nulliparous midtrimester patients was increased by the intra-amniotic instillation of 350 ml Macrodex. There were 2 treatment failures due to the rupturing of the fetal membranes within 2 hours of the instillation. In all the remaining 8 patients the intra-uterine pressure increased gradually following volume increase. However it only reached the magnitude demanded for clinical abortion in 4.

Earlier studies (3–6) demonstrated that an increase of intra-uterine volume (V) in midtrimester pregnant patients provokes an increase in intra-uterine pressure (IUP) and abortion in those instances, where the progesterone (P) levels are low and thus a moderate increase in volume induces a marked increase in the V/P ratio. In normal midtrimester patients a transient and slight increase in volume (induced by isotonic saline) provoked only transient and slight elevation of IUP and failed to induce abortion (7). In the present clinical trials the possibility has been examined that moderate but sustained increase in V by means of a high molecular weight dextran solution, may induce abortion if it provokes the complete evolution of IUP.

MATERIAL AND METHODS

10 nulliparous patients were selected for the study because abortion is less readily induced in nulliparas than in multiparas. The patients were 19 ± 0.5 years old (mean ± S.E.), 19 ± 0.3 week pregnant and had normal (5, 6) plasma P levels (9) (4.29 ± 0.60 ng ml), indicating normal pregnancy. Their estimated gestational age (Naegele) has been confirmed in retrospect by the weight (74 ± 4 g) and crown-heel length (16 ± 1 cm) of the delivered fetuses.

The IUP and oxytocin response was recorded sequentially by the extra-ovular microballoon technique (4), before and at various times after the intra-amniotic instillation of 350 ml Macrodex (Macrodex® Astra AB, Sweden). As a prophylactic measure 800 000 U penicillin with 1 g streptomycin was given intramuscularly. In each recording session first the spontaneous activity and subsequently the uterine response to the intravenous injection of 50 mU oxytocin (OR) was observed for 15 min. Changes in IUP were measured, by calculating (4) the average active pressure (AP mmHg/15 min), the average resting pressure (RP mmHg 15 min), and the average frequency (F number of contractures in 15 min).

Changes in V at 2, 24 and 48 hours after Macrodex instillation were measured in 6 patients by the ultrasound method (using AB-equipment, 4100 MGS, Kretz Technique B-mode scanning 2 MHz crystal). As a contact medium, (between the skin and the crystal) olive oil was used. Moving the crystal over the lower abdomen longitudinally revealed the middle longitudinal section of the uterine outline on the oscilloscope. This image was photographed by a polaroid camera (see the sonograms in Fig. 1 *A–D*). The uterine area was measured by planimetry from the photographs and the uterine volume was calculated from the area.

RESULTS

Of the 10 patients studied, 4 women aborted on the 3rd day after the instillation of Macrodex. Four of the remaining 6 patients who did not abort (within 72 hours of Macrodex instillation), had successful induction of abortion within 24 hours, by means of 20% NaCl. Two of these 4 patients had incipient abortion before saline treatment (as indicated by 1.5 cm cervical dilatation). In 2 patients, the membranes ruptured at 2 and at 3 hours after injection of Macrodex and since they were treatment failures their uteri were emptied by hysterotomy at 24 hours after instillation.

Macrodex increased the uterine area by 70.6%

uterine inseminations were performed to pass the cervical barrier

The results obviously depend on the indications and the fertility status of the couple (7 9) For group I couples the results were generally good. In the literature a conception frequency approaching 100 per cent is reported. In the present series 9 of 10 women conceived and in the exceptional case there were seminal findings which could explain the infertility

For group II couples the frequency of conception reported in the literature varies from 0 to 40 per cent (2) In the present series about one third of the women in group II conceived but miscarriage was very common

The semen quality clearly influenced the rate of conception There were statistically significant differences between those who conceived and those who did not conceive with regard to density percentage of motile spermatozoa, motility degree and for acid phosphatases in seminal plasma. In this series there was one man with extremely small ejaculate volumes but conception occurred after 3 inseminations one man had only 7 million spermatozoa per ml and conception occurred after 4 inseminations. In both cases the sperm motility was good. In cases of oligozoospermia a split ejaculation technic (1 10) has been recommended but this was not used in the present study

Investigation of in vitro sperm penetration in connection with homologous insemination has not been published earlier In the present work groups with high in vitro sperm penetration had significantly higher conception rates than groups with low in vitro sperm penetration No conceptions were produced with semen having an in vitro sperm penetration of less than 6 mm/3 hours and when penetration was in the range 6–19 mm/3 hours the conception rate was lower than when in vitro sperm penetration was 20 mm or more in 3 hours. This is in agreement with an earlier publication (12) where comparison was made between of semen quality in fertile and infertile men.

The postcoital test could not be performed for the couples in group I For group II couples the results of the postcoital test corresponded well with the in vitro sperm penetration test except for some cases where the postcoital test showed poor in vivo penetration because of hostility of the cervical mucus. In cases where both the postcoital test and the in vitro sperm penetration test showed greatly reduced sperm penetration no conceptions were obtained. This finding is of great importance when assessing the prognosis.

In the literature different opinions of the indications for and the technic of homologous insemination are found (4 8) Hanson & Rock (3) state that it is evident that homologous insemination is worthwhile provided ovulation can be detected and collected ejaculates can be improved and properly placed. From the present work it should be concluded rather that careful investigation of the semen properties, including in vitro sperm penetration test, make it possible to judge the prognosis. When the couples are selected with regard to these findings, homologous insemination can be a valuable tool in the treatment of infertility in some cases.

REFERENCES

1 Amelar R. D & Hotchkiss, R. S. *The split ejaculate.* Its use and management of male infertility Fertil Steril *16* 46, 1965
2. Guttmacher A. F The role of artificial insemination in the treatment of sterility Obstet Gynec Surv *15* 767 1960.
3. Hanson, F & Rock, J Artificial insemination with husband's sperm. Fertil Steril 162, 1951
4 Hotchkiss, R. S. & MacLeod, J The distribution of spermatozoa and certain chemical constituents in human ejaculate J Urol 48: 225 1942.
5 Kibrick, S., Belding, D L. & Merril, B Methods for detection of antibodies against mammalian spermatozoa. II. A gelatin agglutination test. Fertil Steril *3* 430 195_
6. Kremer J A simple sperm penetration test. Int J Fertil *10* 209 1965
7 MacLeod, J Gold, R. & McLane C. Correlation of male and female factors in human infertility Fertil Steril 6 112, 1955
8 Payne S. & Skeels, R. Fertility evaluated by artificial insemination. Fertil Steril *5* 32, 1954
9 Rubin, A. Studies in human reproduction. Fertil Steril *1* 581 1961
10 Shields, F Artificial insemination as related to the female Fertil Steril *1* 271 1950
11 Ulstein, M Evaluation of the capillary tube sperm *penetration method for fertility investigations.* Acta Obstet Gynec Scand *51* 285 1972
12. Ulstein, M Sperm penetration of cervical mucus as criterion of male fertility Acta Obstet Gynec Scand 51 335 1972

Submitted fo publication Feb 2 1972

Magnar Ulstein
Fylkessjukehuset Alesund
6017 Asestranda
Norway

Acta Obstet Gynec Scand 52: 9–12, 1973

INDUCTION OF MIDTRIMESTER ABORTION BY INCREASING INTRA-UTERINE VOLUME

M. O. Pulkkinen, P. Liukko, O. Piiroinen and L. Rauramo

From the Department of Obstetrics and Gynecology (Head: Professor L. Rauramo), University of Turku, Turku, Finland

Abstract. The intra-uterine volume of 10 obstetrically normal nulliparous midtrimester patients was increased by the intra-amniotic instillation of 350 ml Macrodex. There were 2 treatment failures due to the rupturing of the fetal membranes within 2 hours of the instillation. In all the remaining 8 patients the intra-uterine pressure increased gradually following volume increase. However, it only reached the magnitude demanded for clinical abortion in 4.

Earlier studies (3, 6) demonstrated that an increase of intra-uterine volume (V) in midtrimester pregnant patients provokes an increase in intra-uterine pressure (IUP) and abortion in those instances, where the progesterone (P) levels are low and thus a moderate increase in volume induces a marked increase in the V/P ratio. In normal midtrimester patients a transient and slight increase in volume (induced by isotonic saline) provoked only transient and slight elevation of IUP and failed to induce abortion (7). In the present clinical trials the possibility has been examined that a moderate but sustained increase in V, by means of a high molecular weight dextran solution, may induce abortion if it provokes the complete evolution of IUP.

MATERIAL AND METHODS

10 nulliparous patients were selected for the study because abortion is less readily induced in nulliparae than in multiparae. The patients were 19.3 0.5 years old (mean S.E.), 14.9 0.3 weeks pregnant and had normal (5, 6) plasma P levels (9) (4.79 0.60 ng/ml), indicating normal pregnancy. Their estimated gestational age (Naegele) has been confirmed in retrospect by the weight (74 ± 4 g) and crown-heel length (16 1 cm) of the delivered fetuses.

The IUP and oxytocin response was recorded sequentially by the extra-ovular microballoon technique (4), before and at various times after the intra-amniotic instillation of 350 ml Macrodex (Macrodex®, Astra AB, Sweden). As a prophylactic measure 800 000 U penicillin with 1 g streptomycin was given intramuscularly. In each recording session first the spontaneous activity and subsequently the uterine response to the intravenous injection of 50 mU oxytocin (OR) was observed for 15 min. Changes in IUP were measured, by calculating (4) the average active pressure (AP mmHg/15 min), the average resting pressure (RP mmHg/15 min), and the average frequency (F number of contractions in 15 min).

Changes in V at 2, 24 and 48 hours after Macrodex instillation were measured in 6 patients by the ultrasound method (using AB-equipment, 4100 MGS, Kretz Technique B-mode scanning 2 MHz crystal). As a contact medium, (between the skin and the crystal) olive oil was used. Moving the crystal over the lower abdomen longitudinally revealed the middle longitudinal section of the uterine outline on the oscilloscope. This image was photographed by a polaroid camera (see the sonograms in Fig. 1 *A–D*). The uterine area was measured by planimetry from the photographs and the uterine volume was calculated from the area.

RESULTS

Of the 10 patients studied, 4 women aborted on the 3rd day after the instillation of Macrodex. Four of the remaining 6 patients who did not abort (within 72 hours of Macrodex instillation) had successful induction of abortion within 24 hours, by means of 20% NaCl. Two of these 4 patients had "incipient" abortion before saline treatment (as indicated by 1.5 cm cervical dilatation). In 2 patients, the membranes ruptured at 2 and at 3 hours after injection of Macrodex and since they were "treatment failures" their uteri were emptied by hysterotomy at 24 hours after instillation.

Macrodex increased the uterine area by 70.6%

uterine inseminations were performed to pass the cervical barrier

The results obviously depend on the indications and the fertility status of the couple (7 9). For group I couples the results were generally good. In the literature a conception frequency approaching 100 per cent is reported. In the present series 9 of 10 women conceived and in the exceptional case there were seminal findings which could explain the infertility

For group II couples the frequency of conception reported in the literature varies from 0 to 40 per cent (2). In the present series about one third of the women in group II conceived but miscarriage was very common.

The semen quality clearly influenced the rate of conception There were statistically significant differences between those who conceived and those who did not conceive with regard to density percentage of motile spermatozoa motility degree and for acid phosphatases in seminal plasma In this series there was one man with extremely small ejaculate volumes but conception occurred after 3 inseminations one man had only 7 million spermatozoa per ml and conception occurred after 4 inseminations. In both cases the sperm motility was good In cases of oligozoospermia a split ejaculation technic (1 10) has been recommended but this was not used in the present study

Investigation of in vitro sperm penetration in connection with homologous insemination has not *been published earlier In the present work groups* with high in vitro sperm penetration had significantly higher conception rates than groups with low in vitro sperm penetration. No conceptions were produced with semen having an in vitro sperm penetration of less than 6 mm/3 hours, and when penetration was in the range 6–19 mm/3 hours the conception rate was lower than when in vitro sperm penetration was 20 mm or more in 3 hours. This is in agreement with an earlier publication (12) where comparison was made between of semen quality in fertile and infertile men

The postcoital test could not be performed for the couples in group I For group II couples the results of the postcoital test corresponded well with the in vitro sperm penetration test except for some cases where the postcoital test showed poor in vivo penetration because of hostility of the cervical mucus. In cases where both the postcoital test and the in vitro sperm penetration test showed greatly reduced sperm penetration no conceptions were obtained. This finding is of great importance when assessing the prognosis.

In the literature different opinions of the indications for and the technic of homologous insemination are found (4 8) Hanson & Rock (3) state that it is evident that homologous insemination is worthwhile provided ovulation can be detected and collected ejaculates can be improved and properly placed. From the present work it should be concluded rather that careful investigation of the semen properties, including in vitro sperm penetration test, make it possible to judge the prognosis. When the couples are selected with regard to these findings, homologous insemination can be a valuable tool in the treatment of infertility in some cases.

REFERENCES

1 Amelar R. D & Hotchkiss, R. S. The split ejaculate. Its use and management of male infertility Fertil Steril *16* 46, 1965
2. Guttmacher A. F The role f artificial insemination in the treatment of sterility Obstet Gynec Surv *15* 767 1960.
3 Hanson, F & Rock J Artificial insemination with husband's sperm. Fertil Steril 2 162, 1951
4 Hotchkiss, R. S. & MacLeod, J The distribution of spermatozoa and certain chemical constituents in human ejaculate J Urol *48* 225 1942.
5 Kibrick, S., Belding, D L & Merril, B. Methods for detection of antibodies gainst mammalian spermatozoa. II A gelatin agglutination test. Fertil Steril *3* 430, 195_
6. Kremer J A simple sperm penetration test. Int J Fertil *10* 209 1965
7 MacLeod, J Gold, R. & McLane C Correlation of male nd female factors in human infertility Fertil Steril *6* 112, 1955
8 Payne S. & Skeels, R. Fertility evaluated by artificial insemination. Fertil Steril *5* 3_, 1954
9 Rubin, A. Studies in human reproduction. Fertil Steril *12* 581 1961
10. Shields, F Artificial insemination as elated to the female Fertil Steril *1* 271 1950
11 Ulstein, M Evaluation of the capillary tube sperm penetration method for fertility investigations Acta Obstet Gynec Scand *51* 285 1972.
1_ Ulstein, M. Sperm penetration f cervical mucus as criterion of male fertility Acta Obstet Gynec Scand *51* 335 1972

Submitted for public tion Feb 2 197

M gnar Ulstei
Fylkessjukehuset Alesund
6017 Asestranda
N r y

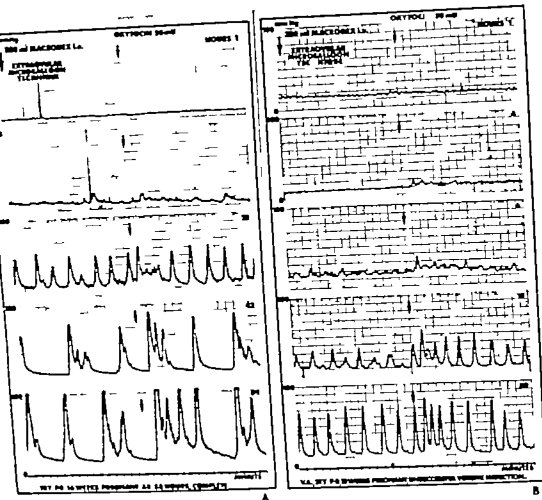


Fig. 3 (A, B) Original recordings (baseline activity and oxytocin test) in successful (A) and nonsuccessful (B) cases of induction by Macrodex instillation in midtrimester pregnancy

mmal AP of 70 mmHg, in spite of rupturing of their membranes spontaneously 8 hours earlier on the average. Fig. 4 also show that those patients who did not abort had at least a partial rise in IUP consistent with the clinical finding of partial ffacement and dilatation of the cervix

One patient complained of abdominal and shoulder pain shortly after Macrode instillation and omited. Her pulse rate increased (to 96 min) and blood pressure decreased (95 mmHg systolic). A pparent leakage of amniotic fluid into the peritoneal ca ity was indicated by decrease in uterine siz after an initial increase

I these 4 patients who successfully aborted af ter Macrodex instillation only the placentae were histologically normal in contrast t those who received additional hypertonic saline treatment.

DISCUSSION

The results show that at around the 15th week of gestation the instillation of 350 ml Macrode lone transiently induced a marked increase in V. At 24–48 hours after treatment the residual V increase is moderate. In spite of this only moderate increase in V the IUP of all the treated patients showed some increase and the cerv xes effaced and dilated ppreciably 1 4 patients the IUP reached the necessary degree for provoking abor tion. There were two treatment failures due to early membrane rupture.

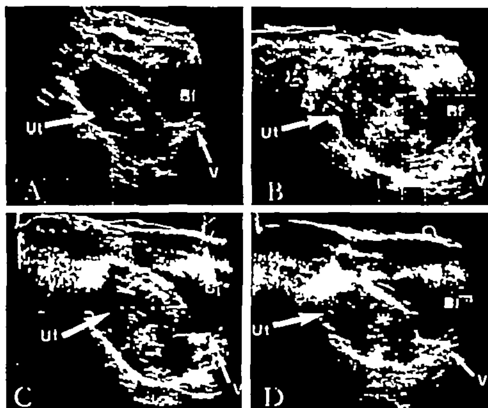
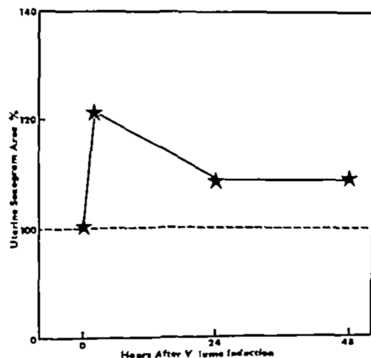


Fig 1 Intra-uterine ohme after intra-amniotic injection of 350 ml Macrodex. Sonograms of the uterus before Macrodex infusion (*A*), h (*B*), 4 h (*C*) and 72 h (*D*) after infusion. Longitudinal scan at midline *Bl* = bladder, *Ut* = uterus, *V* = vagina.

($P < 0.02$) and V by 30%. Fig. 2 illustrates that the marked initial increase in V was only transient and decreased after 2 hours.

Fig. 3 *A* illustrates the IUP and OR of a patient who aborted at 58 hours after Macrodex injection. During the terminal phase of the increase in IUP this patient had an average active pressure exceeding 100 mmHg. Fig. 3 *B* illustrates the limited rise in IUP and OR of a patient who failed to abort within 72 hours.

Fig. 4 illustrates that those 4 patients who aborted after V increase had an average ter



F g 2 Average change of terine ume (measured as areas B-mode sonograms) after ntraamniotic injection of 350 ml Macrodex.

Acta Obstet Gynec Scand 52: 13–22, 1973

THE EFFECT OF PARACERVICAL BLOCK ON CERVICAL DILATATION AND UTERINE ACTIVITY

Helge Jenssen

From the Department of Obstetrics and Gynecology (Head Per Agnar Nilsen), Aker Hospital (Oslo City Hospitals) Oslo, Norway

Abstract. This investigation was carried out to determine whether paracervical block (PCB) has any effect on the duration of the first stage of labour, relating the speed of cervical dilatation to uterine activity. Uterine activity was measured by internal tocometry and calculated in Montevideo units (MU).

Fourty patients were selected and allocated in random manner to PCB or control group, the patients in the latter receiving other kinds of analgesia. Oxytocin was given, when required, to ensure steady and fairly high degree of uterine activity. Four patients were excluded.

The mean duration of the first stage of labour from cervical dilatation 2 to 10 cm, was 161 minutes in the PCB group, 237 minutes in the control group.

Uterine activity calculated as total MU needed for each cm of cervical dilatation, was reduced after PCB, the differences being statistically significant for increments from 3 to 8 cm cervical dilatation inclusive.

PCB caused treatment reduction in frequency of contractions, and relative fall in amniotic pressure between contractions.

It concluded that PCB facilitates cervical dilatation by inhibiting muscular contractions in the lower uterine segment.

The speed of cervical dilatation after paracervical block (PCB) during the first stage of labour is still matter for debate. Several authors state that cervical dilatation is not influenced by PCB (6, 18, 33) others that PCB decelerates the first stage of labour (26, 34). Some have found an accelerating effect of PCB (4, 16, 27, 31).

A PCB when properly administered, renders excellent pain relief in most cases. It is of considerable importance to know exactly its effect on the uterine activity and the speed of cervical dilatation. This knowledge may be important regarding indications and contraindications for its use.

Tocometric studies have been performed using external (31) and internal (37) techniques. It is felt that the accuracy and specificity desired is achieved only by internal registration of amniotic pressure. Due to lack of control groups, earlier investigations do not reveal conclusively whether or not cervical dilatation is influenced by PCB.

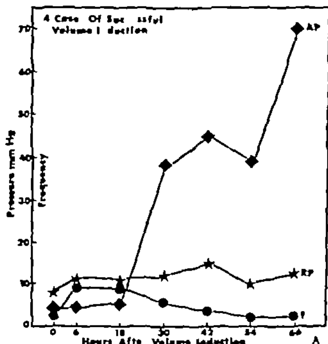
METHODS

Patients

Fourty patients ere selected strictly with the aim of avoiding all factors other than the cervical resistance to uterine activity needed for dilatation. Parturients with an unripe cervix, multiple pregnancy, breech presentation, or suspected mechanical disproportion were excluded. They were then allocated at random either to treatment group receiving PCB at 3 cm cervical dilatation or control group receiving another analgesic at the same stage of cervical dilatation. For reasons arising during the first stage of labour 4 patients among the first 23 were subsequently excluded. In one case more than 5 000 MU were recorded without noticeable progress, and the experiment was abandoned. In one patient only 3 measurements of cervical diameter were made. Occiput posterior position, resulting in operative delivery developed in 2 cases. If the results from these cases had been included in the final analysis, the conclusions would have been unaltered. The last 17 parturients were not allocated to control or treatment groups until the end of the control period. This change of procedure prevented further exclusions after allocation. Table I shows clinical details of patients.

Lindgren found positive correlation between birth weight and uterine activity during first stage of labour (19). Mean infant birth weight was 3 521 g in the PCB group and 3 726 g in the control group. The difference seems to be too small to invalidate the comparisons made between the groups. Mean length of the newborn and circumference of head were virtually identical.

Cervical diameter was assessed by digital examination per vaginam by the author according to the classification of Lindgren (16). These measurements are encumbered



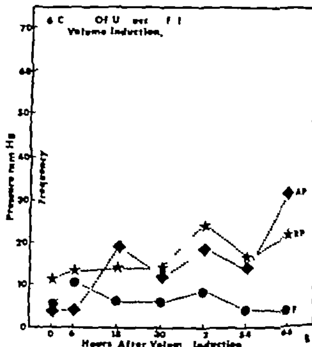


Fig 4 (A B) The average increase of uterine activity after volume induction (active pressure *AP* resting pressure *RP* frequency *F*).

The academic value of this study is the demonstration consistent with the prediction (3) and the earlier finding (6) that increase in V is an effective uterine stimulant. The clinical significance of the study is as yet uncertain. Two patients had early membrane rupture and 4 required subsequent induction with hypertonic saline leaving only 4 successes. A more gradual and extensive V increase may improve the clinical outcome and this possibility is now being examined

The intra amniotic instillation of 200 ml 25% mannitol had a similar (50%) success rate in inducing abortion (1) In contrast, hypertonic saline, when properly administered nearly always provokes abortion (7)

Since the increase in V in the present study was not significantly greater than that induced by hypertonic saline (8) and since hypertonic saline treatment not only increases V but also reduces P (5) it is apparent that a marked increase in the V/P ratio is a better guarantee of the full evolution of IUP and abortion (6) than is a moderate one

ACKNOWLEDGEMENTS

The authors are grateful to Professor A. I. Csapo for suggesting this technique as method of regulating myometrial function to Professor P Laurén for the histological examination of the placentae to Dr K. Willman for the progesterone assays, and to Mrs Marjatta Sotoora for assistance in IUP recording and data analyses.

REFERENCES

1 Craft, I L & Musa, B D. Brit Med J *3* 49 1971
2 Csapo, A. I. Yearbook of Obstetrics and Gynecology (ed. J P Greenhill) Yearbook Medical Publishers, 1966–67
3 Csapo, A. I. *I* Progesterone Its Regulatory Effect on the Myometrium (ed. by G W W Wolstenholme & J Knight), p 13. J & A. Churchill, London, 1969
4 Csapo A I Obstet Gynec Survey *25* 6 515 1970
5 Csapo A I Knobil, E., Pulkkinen, M O Van der Molen, H. J Sommerville I F & Wiest, W G Amer J Obstet Gynec *108* 950, 1970
6 Csapo A I Sauvage J P & Wiest, W G Amer J Obstet Gynec *108* 6. 950 1970
7 Pulkkinen, M O & Kivikoski, A Int J Obstet Gynec *7* 172, 1969
8 — Acta Obstet Gynec Scand *50* 149 1971
9 Willman, K. J Clin Lab Invest Suppl *110* 1?9 1969

Submitted for publication April 1 19

M O Pulkkinen
Naistenklinikk
20520 Turku 5
Finland

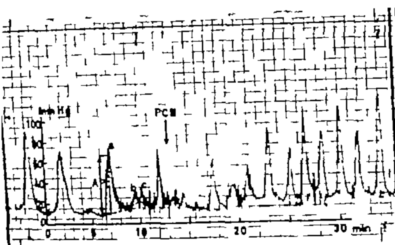


Fig. 2. Amniotic pressure curve, paper speed 1 cm per min. Active pressure, *AP* is measured from the lower margin of tracing, avoiding influence of striated muscle contraction. Contractions and are included in calculations, while *b* is not, as its AP is lower than 10 mmHg. Note narrowing of tracing after PCB. Between 26 and 29 minutes is coupled "bigeminal" contraction. Since the difference between the pressure before and after the two contractions and the pressure between them is less than 10 mmHg, both contractions are included in calculations.

between contractions ("resting tonus") was defined as the lowermost pressure registered between the contractions. The tracing was narrower after PCB than before (Fig. 2), because pain relief abolished most of contractions of voluntary muscles; pressure caused by voluntary muscle contraction was carefully avoided by measuring AP from the lower margin of the tracing. Contractions producing an AP of less than 10 mmHg ere disregarded. Uterine activity in Montevideo units (MU) as calculated as the sum of AP per 10 minutes. The sum of MU for all 10 minute periods taken to dilate the cervix 1 cm was designated as total uterine activity for that dilatation interval.

In some patients, both before and after PCB or other forms of analgesia, 2 or occasionally even more contractions were coupled together. When amniotic pressure between these coupled contractions as more than 10 mmHg above the ordinary level between contractions, only the contraction with the highest AP was included. If the limit between two 10 minute periods divided contraction in about two equal parts, half of the AP value as allocated to each 10 minute period ith the numerical value of half contraction. An unequally divided contraction was allocated to the 10 minute period containing the greater part of

Periods at the end of the control or treatment periods shorter than 3 minutes were ignored, periods of 7 10 minutes duration were reckoned as 10 minute periods, while periods of 3–7 minutes length were increased proportionally to 10 minute period. Such increased periods were approximately evenly dispersed in the two groups.

Analgesia

PCB as given ith modified Kobak needle at 4 points in the spinal lateral fornices, corresponding to 3, 30, 7.30 and 9 clock. Care was taken to deposit the analgesic solution no deeper than 2 3 mm beneath the surface of the mucous membrane, superficial to the pericervical fascia, preferably here the greatest resistance to injection was encountered. This technique limits the local analgesic to the area ith maximal concentration of nerve fibres. By avoiding the loose connective tissue beneath the fascia, so richly supplied with blood vessels, the chance of intravascular injection or rapid absorption (8, 33), is minimized. The only real danger is puncture of the foetal head, safely avoided by inserting finger between the foetal head and outer surface of the cervix, as proposed by Ingelman-Sundberg (11). Paraesthesia of the lower extremity did not occur nor did disturbance of urinary bladder function. Each patient received bupivacaine (Marcain-Adrenalin® Bofors) 0.25% with adrenalin 1 400 000, 4 ml at each point.

The controls received different kinds of analgesics (Table II) at cervical dilatation 3 cm. The quantities given did not seem to change uterine activity to any appreciable tent. All patients were given diazepam (Stesolid® Dumex) 10 mg by m. injection at the start of observations.

Oxytocin

From preliminary experiments as found mandatory for satisfactory results to keep fairly high and steady uterine activity. If uterine activity decreased to level of 120–150 MU oxytocin was administered intravenously or as buccal tablets. In the PCB group oxytocin administration was started in 10 patients before and 4 patients after the block. In the control group 9 patients received oxytocin before, and 5 after the analgesia had been given. In most cases the oxytocin supply as maintained throughout the first stage of labour. The oxytocin given could

Table I *Clinical details of patients*

0 nulliparae. M multiparae

	No. of cases	Age (years mean)	Parity 0	Parity M	Days post expected term (mean)	Admitted for induction	Admitted for spont. labour
PCB group	18	25 7	11	7	3 6	7	11
Control group	18	23 7	13	5	6.5	9	9

with a certain amount of error to have other members of the medical staff perform the assessments proved impracticable. Cervical dilatation was followed with frequent examinations, especially during the latter stages. The diameter was recorded in cm, during contractions, 4-7 times in each patient, at and 3 cm, and at full dilatation, designated as 10 cm diameter The other observations were recorded at different stages of dilatation in order to get relatively long, but irregularly spread intervals. The inaccuracy of assessment is thought to be similar at every examination, and is relatively small if intervals are as great as possible. Special care was taken to get approximately the same distribution of intervals at the start of the treatment period in both groups.

In each group control period with cervical diameter increasing from 2 to 3 cm was observed before administration of analgesia, the phase from cervical diameter 3 cm to full dilatation constituting the treatment period. Each cm of cervical dilatation was designated as one dilatation interval.

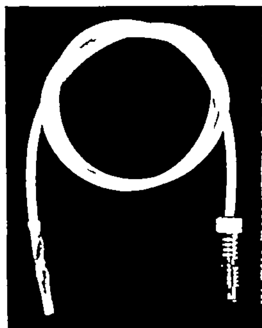


Fig 1 Polyvinyl catheter for recording amniotic pressure, to be passed through cervix. Outer diamete 3 mm, inner diameter 1.8 mm. Note helical slits near tip of catheter diametrically opposed, making the tip more pliable than the rest of the catheter and preventing plugging with vernix.

Measurement of uterine activity

At cervical diameter 2 cm a polyvinyl catheter (Fig. 1) was threaded through the cervix into the amniotic cavity (after amniotomy if membranes were intact) on the *ventral side of the foetal head.* The catheter has an outer/inner diameter of 3/1.8 mm, and an open end as w ll as a helical formed slit, 3 cm long and 1 1.5 mm wide on each side near the tip. This construction has been found to ha e the same advantages as Bengtsson's sponge-tipped catheter (3), having at the same time the advantage of very easy insertion without a guide. Softening of the catheter by the body's heat calls for a rather quick insertion. The slits make the tip more pliable than the rest of the catheter minimizing the risk of foetal or placental injury This model should entirely preclude perforation of the uterine wall, an accident reported ith the use of commercially vailable catheters (10).

The inserted part of the catheter was measured to such length that the tip should be positioned *halfway* between the symphysis pubis and the umbilicus, using this landmark as zero lev l for the transducer A polyvinyl tube with outer inner diameter 1.6–1 mm connected the amniotic catheter by a closed water system to a pressure transducer (Elema-Schönander EMT 35). The signal was passed through an electromanometer (Elema-Schönander EMT 31) with filter set at 70 Hz and recorded on an ink writer (Kompensograph III Siemens). Normally pape speed was 1 cm per minute w th greater disturbances from voluntary muscle contractions the speed was increased t facilitate correct reading of active pressure

Before starting each xperiment the system as calibrated against column of mercury in such a ay that 100 mmHg gave 10 cm deflection f the Kompensograph pen. During the experiment the setting of th system was frequently controlled w th a built in lectrical calibration device.

Calculations

Number of contractions pe 10 minutes was read from the pressure curve Strength of each contraction, acti e pressure AP was measured along the perpendicular from th highest point of each contraction to th straight line connecting the lev l f amniotic pressure bef re and after the contraction, in mmHg (Fig. 2). Amniotic pressure

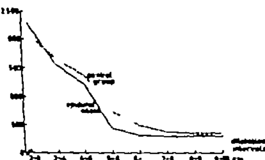


Fig. 4. Mean total uterine activity of 3 patients given epidural block (Mercaso-Adrenalin 0.25% © Bofors) at cervical dilatation 3 cm. The values of the control group taken from Table VII.

The cervical muscle fibres have been shown to exert a radial as well as a tangential pressure on the presenting part (18, 21). It is believed that the anaesthetic solution injected by PCB inactivates the muscle cells of the cervix and lower uterine segment, thereby augmenting the difference in force of contraction between the upper and lower part of the myometrium. Inactivation of the lower uterine segment strengthens the triple descent gradient of Alvarez & Caldeyro-Barcia (1) characterizing the normal labour contraction.

Csapo has shown in vitro that the tension developed by stimulation of a myometrial muscle strip diminishes as decreasing length of it is stimulated (7). Applying these results to the human uterus in vivo one would predict a fall of amniotic pressure during contractions after PCB. This does not take place (Table V) because the effect of PCB exerts itself pharmacologically in the wall of the uterus, i.e. in the second power while it works mechanically via the third power. The diminished tension thereby cannot be recorded using the technique described here.

Narik has shown that during each uterine contraction, fluid is displaced from the upper to the lower part of the amniotic cavity causing a bulging of the lower uterine segment (25). The volume of the lower uterine segment temporarily increases at each contraction. Lindgren has been able to confirm this convincingly by pressure measurement (20). A pressure transducer located 1 cm above the equator of the foetal head during the initial phase of contraction registered the high mechanical pressure between cervix and foetal head, but at the peak of contractions it registered the lower hydrostatic pressure of the amniotic cavity.

When wall tension in the lower uterine segment has decreased because of inactivation of the muscle fibres by PCB, resistance to dilatation from the fluid shift is diminished. The lower uterine segment will yield more easily, resulting in an increased bulging. By rough approximation the lower uterine segment might be considered as part of a sphere. Let r_1 be its radius; by applying the law of Laplace (24)

$$T_1 = \frac{r_1}{2} P$$

or written

$$\frac{T_1}{r_1} = \frac{P}{2}$$

it is seen that the wall tension of the lower uterine segment, T_1, will increase when r_1 augments, even if amniotic pressure, P, remains unchanged (Table V). Augmented wall tension during contraction will promote dilatation.

Increased fluid shift after PCB also will result in a shorter radius r_u of the upper part of the uterus after PCB than before. By approximation the upper part of the uterus as well may be considered as part of a sphere. If wall tension here at maximal contraction is T_u, then from the law of Laplace

$$\frac{T_u}{r_u} = \frac{P}{2}$$

it is seen that T_u may decrease when r_u shortens, with amniotic pressure P remaining on the same level (Table V). Thus the in vitro findings of Csapo (see above) may be valid for the changes brought about by PCB in the human uterus in vivo.

Mode of PCB action in lower uterine segment inactivation

Injection of an inert solution (NaCl) seems to be without influence on cervical dilatation (34, 37). Since PCB without vasoconstrictor also reduces uterine activity (37), the local analgesic must be of primary importance. X-ray contrast injected paracervically spreads to the tissues surrounding the lower uterine segment (15). The local analgesic after PCB in the same manner will come in

Table VII *Total uterine activity for each dilatation interval treatment period, and first stage of labour (MU mean)*

	Dilatation intervals, cm									
	2 3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	3–10	10
PCB group	2 469	59	519	352	315	302	324	311	718	5 186
Control group	2 106	1 662	1 375	628	504	383	365	351	5 365	7 372
p	> 0 1	< 0.0005	< 0.0005	< 0.025	< 0.01	> 0.05	> 0.2	> 0.2	< 0.0005	< 0 0005

Calculated as sum of MU of all 10 minute periods for each dilatation interval.

one minute Apgar scores of 6 points, all others had scores of 8 points or higher Five minute Apgar scores were 9 or 10 points.

There was no difference in post partum blood loss between the groups. No case of infection be cause of the relatively frequent vaginal examina tions or the PCB was encountered.

DISCUSSION

This investigation does not reveal any discernible alteration in the dynamics of uterine contrac tions brought about by PCB The mean AP and mean uterine activity (Tables V and VI) do not change significantly after the block. Notwithstanding this fact, duration of the first stage of labour is shortened in the PCB group at the same time total uterine activity measured in MU

Table VIII. *Total uterine activity and parity (MU mean)*

		No. of patients	Dilatation intervals, cm 2 3	3–10
PCB group	nulliparae	11	2 468	2 989
	multiparae	7	2 469	2 291
Control group	nulliparae	13	2 308	5 574
	multiparae	5	1 825	4 822

Calculation as for Table VII

Table IX *Amniotic pressure between contractions (mmHg mean)*

	2 3	3–4	4–5	5–6	6–7	7–8	8–9	9–10
PCB group	*17.8*	*17.5*	*16.5*	*16.3*	*15 4*	*15.–*	*15.9*	*16 7*
Control group	12.1	1–.3	12.8	14 6	14.1	*13.5*	*13 4*	*13 7*

is reduced compared with the control group (Table VII) It should be emphasized that at least the start and end of the control period, as well as the end of the treatment period, are quite exactly determined. The only explanation of these findings is that cervical dilatation is relieved by PCB

Cause of enhanced cervical dilatation after PCB

The dissociation of pain relief and the enhanced cervical dilatation suggest that pain relief *per se* is not the cause.

In spite of complete pain relief in 3 cases of epidural anaesthesia during the first stage of labour cervical dilatation was not promoted (Fig 4) Those cases, of whom 2 were multiparae behaved principally as the control cases.

The uterine cervix contains smooth muscular tissue even at term (30) As shown by many authors, this muscular tissue is capable of contraction during labour (5 14 18 29) opposing the dilatant effect of the rest of the myometrium.

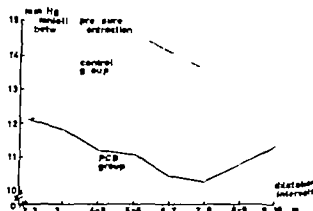


Fig 3 Amniotic pressure between contractions Value of PCB group during control period set equal to that of the control group, the other values of the PCB group calculated relative to this.

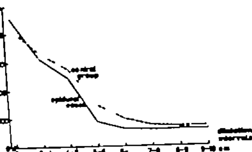


Fig. 4. Mean total uterine activity of 3 patients given epidural block (Marcain-Adrenalin 0.25% ® Bofors) at cervical dilatation 3 cm. The values of the control group taken from Table VII.

The cervical muscle fibres have been shown to exert a radial as well as a tangential pressure on the presenting part (18, 21). It is believed that the anaesthetic solution injected by PCB inactivates the muscle cells of the cervix and lower uterine segment, thereby augmenting the difference in force of contraction between the upper and lower part of the myometrium. Inactivation of the lower uterine segment strengthens the triple descent gradient of Alvarez & Caldeyro-Barcia (1) characterizing the normal labour contraction.

Csapo has shown in vitro that the tension developed by stimulation of a myometrial muscle strip diminishes as decreasing length of it is stimulated (7). Applying these results to the human uterus in vivo one would predict a fall of amniotic pressure during contractions after PCB. This does not take place (Table V) because the effect of PCB asserts itself pharmacologically in the wall of the uterus, i.e. in the second power while it works mechanically in the third power. The diminished tension thereby cannot be recorded using the technique described here.

Narik has shown that during each uterine contraction, fluid is displaced from the upper to the lower part of the amniotic cavity causing bulging of the lower uterine segment (25). The volume of the lower uterine segment temporarily increases at each contraction. Lindgren has been able to confirm this convincingly by pressure measurements (20). A pressure transducer located 1 cm above the equator of the foetal head during the initial phase of contraction registered the high mechanical pressure between cervix and foetal head, but at the peak of contractions it registered the lower hydrostatic pressure of the amniotic cavity.

When wall tension in the lower uterine segment has decreased because of inactivation of the muscle fibres by PCB, resistance to dilatation from the fluid shift is diminished. The lower uterine segment will yield more easily, resulting in an increased bulging. By rough approximation the lower uterine segment might be considered as part of a sphere. Let r_1 be its radius; by applying the law of Laplace (24)

$$T = \frac{r_1}{2} P$$

or written

$$\frac{T_1}{r} = \frac{P}{2}$$

it is seen that the wall tension of the lower uterine segment, T, will increase when r_1 augments, even if amniotic pressure, P, remains unchanged (Table V). Augmented wall tension during contraction will promote dilatation.

Increased fluid shift after PCB also will result in a shorter radius, r_u, of the upper part of the uterus after PCB than before. By approximation the upper part of the uterus as well may be considered as part of a sphere. If wall tension here at maximal contraction is T_u, then from the law of Laplace

$$\frac{T}{\;} = \frac{P}{2}$$

it is seen that T may decrease when r shortens, with amniotic pressure, P, remaining on the same level (Table V). Thus the in vitro findings of Csapo (see above) may be valid for the changes brought about by PCB in the human uterus in vivo.

Mode of PCB action in lower uterine segment inactivation

Injection of an inert solution (NaCl) seems to be without influence on cervical dilatation (34, 37). Since PCB without vasoconstrictor also reduces uterine activity (37), the local analgesic must be of primary importance. X-ray contrast injected paracervically spreads to the tissues surrounding the lower uterine segment (15). The local analgesic after PCB in the same manner will come in

contact with rather large parts of the lower uterine segment. Supposing that half the injected amount of 40 mg bupivacaine is absorbed into the blood stream while the rest diffuses to say 1 000 ml tissue the tissue concentration for some time would be about 20 μg per ml. As 10–20 μg/ml mepivacaine which is a less potent local analgesic than bupivacaine has been shown to produce atrioventricular block in midterm isolated foetal hearts (2) this concentration most likely is sufficiently high to block the electrical impulse accompanying each contraction (7 35) or the *propagation of the myometrial contraction wave* (1 5)

The in vitro effect of local analgesics has been tested on human (22) and animal myometrium (12 32) The results seem ambiguous, and vary with experimental conditions. Moreover strip preparations undergo structural changes resulting in altered functional properties (24)

Adrenalin was shown by Lindgren (18) to abolish contraction in the cervical region. It stimulates both α and β receptors in the myometrium near term the effect on the latter predominating (36) β receptor stimulation may explain the effect of adrenalin, and ought to be one of the indications for its addition to the solution even if this does not seem to prolong the effect of the analgesic solution to any great extent (34)

Amniotic pressure between contractions

This parameter was significantly higher during the control period in the PCB group than among the controls (Table IX) The shape of the pressure tracings precludes factors outside the uterus having caused this difference Also difference in tension of the uterine wall in these randomly selected groups is unlikely Even if the transducer in most cases was levelled by sight the distance of the transducer from the patient was kept as short as possible making inaccurate levelling of the transducer an *unlikely reason for this* mean difference of 7 8 cm H_2O

Movements of the patients in bed is another possible explanation However in only one patient of the control group at cervical dilatation from 5 to 7 cm was the transducer not corrected in position after such movements.

Most likely the difference in amniotic pressure between contractions during the control period was due to a difference in abdominal shape of the patients. Usually the most ventral point of *the uterus was at or near the umbilicus, but in* some patients the umbilical level was dorsal (and caudal) to the most ventral point of the uterus. In such a patient there will be an increase in amniotic pressure equal to the height of a water column between the most ventral point of the uterus and the umbilical level. Movement of the catheter tip during cervical dilatation is not believed to be of practical significance with the applied technique.

Jung et al (13) with their external technique found a transitory hypertonic uterine activity in 68 9% of their patients after PCB which stands in contrast to the results of Zourlas & Kumar (37) and the present investigation (Fig. 3) It must be questioned whether resting tension of the uterine wall which is the most important factor deciding amniotic pressure between contractions, should be derived from external tocographs.

Other remarks

Frequency of contractions normally increases during the first stage of labour if pethidine or no analgesics are given (8 17) The control group did not show this increase possibly because administration of oxytocin was often initiated in the control period. Using internal tocography Zourlas & Kumar (37) found decreased uterine activity after PCB in half of their 43 patients, due to either *lowered frequency or fall in AP* The present series showed a decrease of frequency (Table IV) Deliberate application of oxytocin is probably the cause of unchanged AP (Table V) Jung et al. (13) found increased frequency of contractions in 60% and decreased frequency in only 7 4% of their 413 patients after PCB They used external tocography rendering calibration and thereby definition of a labour contraction, impossible Under such *conditions especially a control group is very necessary*

Preliminary experiments showed that the facilitation of cervical dilatation by PCB was not so easily demonstrated if the block was administered at a cervical dilatation of 5 cm or more At a cervical dilatation of 3 cm at least the nulliparae are in the acceleration phase of Friedman (9) It is believed that maximum benefit, regarding cervi-

cal dilatation is derived from PCB by application at this relatively early stage. To ensure that conditions are favourable is most important. In this series one patient had to be excluded because of lack of engagement of the foetal head.

Apart from the analgetic effect of PCB cervical dystocia should be regarded as an indication for its use. Inhibiting active muscle contraction in the lower uterine segment, PCB probably diminishes the strain on a scar from previous caesarean section. PCB therefore may be administered with prudence in such cases.

REFERENCES

1. Alvarez, H. & Caldeyro-Barcia, R. The normal and abnormal contractile waves of the uterus during labour. Gynaecologia (Basel) *138*, 190, 1954.
2. Andersson, K.-E., Ingemarsson, I. & Nilsson, E. Contractility of isolated human foetal hearts. Influence of contraction rate, acid-base parameters and local anaesthetic. Acta Physiol Scand, suppl. *353* 1–47 1970.
3. Bengtsson, L. Ph. The sponge-tipped catheter—a modification of the open-end catheter for recording of myometrial activity in vivo. J Reprod Fert *16*: 115 1968.
4. Brown, E. O. Engel, T. & Douglas, R. G. Paracervical block analgesia in labor. Obstet Gynec (NY) *26* 195 1965.
5. Caldeyro Barcia, R. & Alvarez, H. J Obstet Gynec Brit Emp *59* 646, 1952.
6. Cooper, K. & Moir, J. Ch. Paracervical nerve block. A simple method of pain relief in labour. Brit Med J *1* 1372, 1963.
7. Csapo, A. I. Smooth muscle as a contractile unit. *I* Proceedings of a symposium on vascular muscle. Physiol Rev suppl. *5* 7–33, 1962.
8. DeVoe, S. J. DeVoe, J. K. Rigsby, W. C. & McDaniels, Betty A. Effect of meperidine on uterine contractility. Amer J Obstet Gynec *105* 1004, 1969.
9. Friedman, E. A. Labor. Clinical Evaluation and Management, pp. 27–41. Appleton-Century-Crofts, New York 1967.
10. Haverkamp, A. & Bowes, Jr. W. A. Uterine perforation. A complication of continuous fetal monitoring. Amer J Obstet Gynec *10* 667 1971.
11. Ingelman-Sundberg, A. Praktiska synpunkter på paracervikalblockad. Sv Läkartidn *67* 318, 1970.
12. Jindal, M. N. & Patel, M. A. Oxytocic effect of lignocaine and its recently synthesized analogues. Ind J Physiol Pharmacol *9* 167 1965.
13. Jung, H. Kopecky, P. & Klöck, F. K. Die fetale Gefährdung durch die Paracervikalblockaden. Geburtsh Frauenheilk *29* 519 1969.
14. Karlson, S. On the motility of the uterus during labour and the influence of the motility pattern on the duration of the labour. Acta Obstet Gynec Scand *28* 209 1949.
15. Kobak, A. J. Sadove, M. S. & Mazzola, W. T. Anatomic studies of transvaginal regional anesthesia. Roentgenographic visualization of neural pathways. Obstet Gynec (NY) *19* 302, 1962.
16. Kobak, A. J. & Sadove, M. S. Combined paracervical and pudendal nerve blocks—a simple form of transvaginal regional anesthesia. Amer J Obstet Gynec *81* 72, 1961.
17. Kripold, A. J. Myers, G. G. & Caldeyro-Barcia, R. Uterine contractions in spontaneous labor. Amer J Obstet Gynec *106*. 378 1970.
18. Lindgren, L. The lower part of the uterus during the first stage of labour in occipito-anterior vertex presentation. Studies by means of intrauterine tocography. Acta Obstet Gynec Scand *34* suppl. 2: 1–79 1955.
19. — Die Einwirkung der Grösse des Fetus auf die normale Eröffnung der Cervix. Arch Gynäk *194* 460, 1961.
20. — The engagement of the foetal head in the uterus when the vertex presents. Acta Obstet Gynec Scand *51* 37 1972.
21. Lindgren, L. & Smyth, H. Cervical tension during labor. Amer J Obstet Gynec *95* 414 1966.
22. McGaughey, H. S. Corey, E. L., Eastwood, D. & Thornton, W. N. Effect of synthetic anesthetics on the spontaneous motility of human uterine muscle in vitro. Obstet Gynec (NY) *19* 233 1962.
23. Marando, C. L'anestesia paracervicale in travaglio di parto. Arch Ostet Ginecol (Napoli) *73* 537 1968.
24. Mosler, K. H. The dynamics of uterine muscle. *I* Bibliotheca Gynaecologica, No. 48. Advances in Obstetrics and Gynaecology (ed. A. Rossi), S. Karger Basel and New York, 1968.
25. Nixon, G. The method of transfer of labour contractions to the contents of the uterus. J Obstet Gynaec Brit Emp *66* 38, 1959.
26. Pitkin, R. M. & Goddard, W. B. Paracervical and uterosacral block in obstetrics—a controlled, double-blind study. Obstet Gynec (NY) *21* 737 1963.
27. Ramsey, R. Paracervical block for first-stage pain in primigravidas. Obstet Gynec (NY) *27* 757 1966.
28. Sandmire, H. F. & Austin, S. D. Paracervical block anesthesia in obstetrics. J Amer Med Ass *187* 775, 1964.
29. Schild, H. O. Fitzpatrick, R. J. & Nixon, W. C. W. Activity of the human cervix and corpus uteri. Their response to drugs in early pregnancy. Lancet *1* 250, 1951.
30. Schwalm, H. & Dubrauszky, V. The structure of the musculature of the human uterus—muscle and connective tissue. Amer J Obstet Gynec *94* 391, 1966.
31. Simonsen, M. Paracervikal blokade. Ugeskr Laeg *129* 1108 1967.
32. Ushansky, M. The effect of procaine on the uterine contractility. Akusherstvo i Ginekologiia (Mosk.) *4* 17 1966.
33. Van Praagh, I. G. L. & Povey, W. G. Paracervical block anaesthesia in labour. Canad Anaes Soc J *14* 222, 1967.
34. Westholm, H., Magno, R. & Berg, A. A. Experiences with paracervical block. A double-blind study with

contact with rather large parts of the lower uterine segment. Supposing that half the injected amount of 40 mg bupivacaine is absorbed into the blood stream while the rest diffuses to say 1 000 ml tissue th tissue concentration for some time would be about 20 / g per ml. As 10–20 μg/ml mepivacaine which is a less potent local analgesic than bupivacaine has been shown to produce atrioventricular block in midterm isolated foetal hearts (2) this concentration most likely is sufficiently high to block the electrical impulse accompanying each contraction (7 35) or the propagation of the myometrial contraction wave (1 5)

The in vitro effect of local analgesics has been tested on human (22) and animal myometrium (12, 32) The results seem ambiguous, and vary with experimental conditions. Moreover strip preparations undergo structural changes resulting in altered functional properties (24)

Adrenalin was shown by Lindgren (18) to abolish contraction in the cervical region It stimulates both α and β receptors in the myometrium near term the effect on the latter predominating (36) β receptor stimulation may explain the effect of adrenalin and ought to be one of the indications for its addition to the solution, even if this does not seem to prolong the effect of the analgesic solution to any great extent (34)

Amniotic pressure between contractions

This parameter was significantly higher during the control period in the PCB group than among the controls (Table IX) The shape of the pressure tracings precludes factors outside the uterus having caused this difference Also difference in tension of the uterine wall in these randomly selected groups is unlikely Even if the transducer in most cases was levelled by sight, the distance of the transducer from the patient was kept as short as possible making inaccurate levelling of the transducer an unlikely reason for this mean difference of 7 8 cm H_2O

Movements of the patients in bed is another possible explanation However in only one patient of the control group at cervical dilatation from 5 to 7 cm was the transducer not corrected in position after such movements.

Most likely the difference in amniotic pressure between contractions during the control period was due to a difference in abdominal shape of the patients. Usually the most ventral point of the uterus was at or near the umbilicus, but in some patients the umbilical level was dorsal (and caudal) to the most ventral point of the uterus. In such a patient there will be an increase in amniotic pressure equal to the height of a water column between the most ventral point of the uterus and the umbilical level. Movement of the catheter tip during cervical dilatation is not believed to be of practical significance with the applied technique

Jung et al. (13) with their external technique found a transitory hypertonic uterine activity in 68 9% of their patients after PCB which stands in contrast to the results of Zourlas & Kumar (37) and the present investigation (Fig. 3) It must be questioned whether resting tension of the uterine wall, which is the most important factor deciding amniotic pressure between contractions, should be derived from external tocographs.

Other remarks

Frequency of contractions normally increases during the first stage of labour if pethidine or no analgesics are given (8 17) The control group did not show this increase, possibly because administration of oxytocin was often initiated in the control period. Using internal tocography Zourlas & Kumar (37) found decreased uterine activity after PCB in half of their 43 patients, due to either lowered frequency or fall in AP The present series showed a decrease of frequency (Table IV) Deliberate application of oxytocin is probably the cause of unchanged AP (Table V) Jung et al. (13) found increased frequency of contractions in 60% and decreased frequency in only 7 4% of their 413 patients after PCB They used external tocography rendering calibration and thereby definition of a labour contraction impossible Under such conditions especially a control group is very necessary

Preliminary experiments showed that the facilitation of cervical dilatation by PCB was not so easily demonstrated if the block was administered at a cervical dilatation of 5 cm or more At a cervical dilatation of 3 cm at least the nulliparae are in the acceleration phase of Friedman (9). It is believed that maximum benefit regarding cervi-

Acta Obstet Gynec Scand 52: 23–24 1973

THE EFFECTS OF AN ORAL COMBINED CONTRACEPTIVE ON PLASMA LEVELS OF GLUCOSE, FREE FATTY ACIDS GLYCEROL, D-β HYDROXYBUTYRATE AND TRIGLYCERIDES

N O Lunell, B Persson and G Ohqvist

From the Department of Obstetrics and Gynecology (Head: Professor A. Ingelman-Sundberg), Sabbatsberg Hospital, Karolinska Institute and the Department of Pediatrics (Head: Professor R. Zetterström), St. Görans Hospital, Karolinska Institute Stockholm, Sweden

Abstract The effects of an oral combined contraceptive (OC) (norethisterone + mestranol) on plasma levels of glucose free fatty acids (FFA), glycerol, D-β-hydroxybutyrate and triglycerides in 39 healthy women were followed. Plasma samples were collected before and during the end of the first and second cycles of OC administration. OC caused a significant increase in triglycerides while the levels of glucose, FFA, glycerol and D-β-hydroxybutyrate were unaffected.

Combined oral contraceptives (OC) have been shown to cause elevated levels of fasting plasma triglycerides (3 11 12 for ref also see 8).

Although the precise mechanism behind the elevation of plasma triglycerides remains unknown it seems that it is related to the oestrogen content of the OC preparation (12). It has been suggested that the increased levels of triglycerides could be explained by a reduced removal rate due to a decrease in tissue lipoprotein lipase activity as indicated by a reduced post-heparin lipolytic activity (2 11)

However the removal rate of intravenously administered fat from the circulation is unaffected by OC-therapy (11) Recent turnover studies also indicate that OC-therapy causes an enhanced inflow of triglycerides to the blood, i.e. increased hepatic synthesis and release of triglycerides (3) Contradictory results have been reported concerning the possible diabetogenic effects of different combinations of oral contraceptive preparations (8)

Some of these studies have shown that OC treatment could cause glucose intolerance and elevation of plasma insulin and free fatty acid (FFA) concentrations.

The present study was undertaken to find out if a combined OC preparation (norethisterone + mestranol) could affect lipid mobilization and ketone body production as indirectly reflected in changes in plasma levels of glycerol, FFA and D-β-hydroxybutyrate.

MATERIAL AND METHODS

A total of 39 healthy women volunteered to take part in the study All women were within 10% of the ideal body weight and none of them had a family history of diabetes. The mean values (± S.E.M.) for age, height, and pre-treatment weight were 25.1 ± 1.0 years, 165.6 ± 1.1 cm, and 58.3 ± 1.2 kg respectively Overnight fasting venous blood was drawn at 8.00 a.m. before treatment and during the end of the first (= 39) and second ($n = 26$) cycle of treatment with Conlunett® (norethisterone 1 mg + mestranol 0.1 mg). The plasma samples were analysed for glucose (glucose oxidase AB KABI, Stockholm, Sweden), glycerol (5), FFA (6), triglycerides (4), D-β-hydroxybutyrate (9), alkaline phosphatase (7), glutamic-oxaloacetic transaminase activity (GOT) (10) and glutamic-pyruvic transaminase activity (GPT) (10).

Statistical differences between values obtained before and during treatment were calculated by means of the Student's paired t-test.

RESULTS

The plasma triglyceride level is increased significantly ($p < 0.05$) after 1 month of treatment and the levels remained elevated and unchanged during the second month of treatment (see Table 1). The fasting values of glucose glycerol FFA and D-β-hydroxybutyrate were not significantly influenced statistically by the treatment (see Table 1). Nor were any changes observed in alkaline phos-

bupivacaine (Marcaine®). Acta Obstet Gynec Scand 49 335 1970.

35 Wolfs, G Leeuwen, M van, Rottinghuis, H. & Boeles, J Th. F., An electromyographic study of the human uterus during labor Obstet Gynec (NY) 37 241 1971

36. Wood, C., Myometrial contractility in pregnancy and labour In Modern Trends in Obstetrics (ed. R. J Kellar), vol. IV pp 64–68, Butterworths, London, 1969

37 Zourlas, P A. & Kumar D An objective evaluation of paracervical block on human uterine contractility Amer J Obstet Gynec 91 17 1965

Submitted fo publicatio June 5 197

Helge Jenssen
Department of Gynaecology
Aker Sykehus
Oslo 5
Norway

Acta Obstet Gynec Scand 52: 25–28 1973

ON THE PASSAGE OF AN EXOGENOUS DIAMINE OXIDASE INHIBITOR TO THE AMNIOTIC FLUID

Åke Törnqvist, Anne-Marie Fredholm, Fredrik Jonassen and Göran Samsioe

From the Department of Obstetrics and Gynecology (Head: Prof. Sam Brody) and the Department of Clinical Chemistry (Head: Prof. S. en Lindstedt), University of Gothenburg, Gothenburg, Sweden

Abstract. In a previous study on diamine oxidase activity of amniotic fluid (4) it was questioned whether enzyme inhibition with an intramuscular injection of aminoguanidine sulphate was due to a passage of the inhibitor to the amniotic fluid or was secondary to enzyme inhibition in the maternal blood. In the present investigation amniotic fluid was sampled from 23 cases in the second trimester of gestation. In 15 cases, aminoguanidine sulphate was given 1 hour before sampling. In 5 cases aminoguanidine sulphate was detected in the amniotic fluid by thin layer chromatography and a subsequent staining technique. In 10 cases the presence of a diamine oxidase inhibitor in the amniotic fluid was shown by observing the effect of this amniotic fluid on venous plasma with a high diamine oxidase activity. When aminoguanidine sulphate was not given, the amniotic fluid did not show such inhibitory effects. Nor did the incubation itself inhibit the enzyme.

In a previous study on diamine oxidase activity during pregnancy (4) the enzyme activity of amniotic fluid was shown to be significantly lower in women who received aminoguanidine sulphate (a diamine oxidase inhibitor) intramuscularly than in non-treated subjects. Whether the low enzyme activity was due to a passage of the inhibitor to the amniotic fluid or was secondary to an inhibitor of diamine oxidase in the maternal plasma was uncertain. To study the possible presence of aminoguanidine in the amniotic fluid after an intramuscular injection, two procedures have been used.

1. Thin layer chromatography and subsequent staining to show spots of aminoguanidine.

2. Incubation of amniotic fluid with pregnant venous plasma to show a decrease in enzyme concentration of the mixture.

MATERIAL AND METHODS

Five volunteers in the second trimester of pregnancy admitted to the clinic for legal abortion were given 1 mg of aminoguanidine sulphate per kg of body weight intramuscularly. One hour later amniotic fluid was sampled by puncture through the abdominal wall. After centrifugation the supernatant was adjusted to pH 5 with 1 M acetic acid and heated to 90°C for denaturation of protein. After a second centrifugation the supernatant still showed small amounts of protein according to the sulphosalicylic acid test. The supernatant was freeze-dried and dissolved in 5 ml 0.5 M acetic acid and heated to 60°C in a water bath for 20 minutes. Undissolved material was allowed to sediment and the still warm solution was taken to thin layer chromatography (TLC). All samples had a pH of approximately 2.5. The glass plates (Thermoplas AB) used for TLC measured 25 × 25 cm and were covered with silica gel according to Stahl (2). After activation at 110°C for 1 hour a one-dimensional chromatography was run in an n-butanol/acetic acid/water (4 : 1 : 1) system for 6 hours. Sample volumes ranging from 10 to 200 µl were applied in spots not exceeding 1 cm in diameter along with reference solutions. After drying, the plates were sprayed with alphanaphthol-diacetyl reagent or pentacyano-aquoferrate in acetone-water (1 : 1) (PCF-reagent), both made up according to Smith (1). Aminoguanidine was best detected when spots containing 100 µl were used.

Amniotic fluid was also sampled from 18 other abortion cases in the second trimester of pregnancy. Ten of these women received aminoguanidine intramuscularly as earlier described. After centrifugation, one part of the amniotic fluid was stored at −20°C. Amniotic fluid was also incubated with pregnant venous plasma, in proportions of 3 to 1. The venous plasma was in most cases sampled separately in some of the cases. After incubation in a water bath at 37°C for 1 hour all samples were stored at −20°C and analysed for diamine oxidase activity by means of a radioassay procedure (3) as soon as possible.

With known initial concentrations of diamine oxidase in

Table I *Mean values (±S E.M.) of glucose glycerol FFA D-β-hydroxybutyrate and triglycerides before and after 1 and 2 months of treatment with Conlumette R*

	Glucose (mg/100 ml)	Glycerol (mM)	FFA (mM)	D-β-hydroxybutyrate (mM)	Triglycerides (mM)
Before treatment	74 6±2.0	0.089±0.011	0.45±0 05	0.079±0.011	0.58±0.04
After 1 month of treatment	75.8±1.8	0.087±0.014	0.47±0.05	0.108±0.024	0.75±0.06
After 2 months of treatment	72.4±3.3	0.062±0.007	0.45±0.04	0.097±0 017	0.71±0.07

phatases, GOT or GPT during treatment. There was no significant correlation between the paired values of FFA and triglycerides before or during treatment. Highly significant correlations ($p <$ 0 001) were found between glycerol and FFA values before and after 1 month of treatment *($r = 0.54$ and 0 69 respectively)* and also between FFA and D-β hydroxybutyrate ($r = 0$ 73 and 0 65 respectively)

COMMENTS

The present findings of elevated fasting plasma triglycerides and unchanged plasma FFA con centrations are in agreement with previous stud ies (3 11 12, for ref see also 8) The results also indicate that the oestrogen containing OC pre paration used did not significantly influence lipid mobilization as indirectly reflected in unchanged fasting plasma glycerol levels. The hepatic uptake of FFA is proportional to the arterial concentra tion of FFA (1) The present observation of a positive correlation between plasma FFA and D-β-hydroxybutyrate concentrations are in agreement with previous findings (9 13) which in directly suggest that the plasma FFA concentra tion is one factor controlling hepatic ketogenesis. The production of ketone bodies is also related to the rate of carbohydrate utilization. In view of the unchanged and normal plasma concentrations of D-β-hydroxybutyrate and glucose it is unlikely that the OC treatment given has caused any sig nificant impairment of carbohydrate metabolism Since the present study only deals with changes occurring in overnight fasting values the possibility cannot be excluded that a prolonged fast ing period during OC treatment might exacerbate the metabolic effects of OC.

ACKNOWLEDGEMENTS

The investigation has been supported by the S edish Diabetes Association and the Swedish Medical Research Council 13 P 3268.

REFERENCES

1 Fine M. B. & Williams, R. H Am J Physiol 199 403 1960.
2. Hazzard, W R., Spiger M. J Bagdade J D & Bierman, E. L. New Engl J Med 80 471 1969
3 Kekki, M & Nikkilä, E. A.. Metabolism 20 878, 1971
4 Laurell, S.. Scand J Clin Lab Invest 18, 668, 1966.
5 Laurell, S. & Tibbling, G Clin Chim Acta 13 317 1966.
6. — Clin Chim Acta 16 57 1967
7 Laurent, B. & Norberg, B Scand J Clin Lab Invest 12 154 1960.
8. Lunell, N O & Persson, B., In Current Problems in Fertility (ed. A. Ingelman-Sundberg & N O. Lunell) p. 170. Plenum Press, New York, 1971
9 Persson, B. Scand J Clin Lab Invest 25 9 1970.
10. Reitman, S. & Frankel, S. Am J Clin Pathol 28 56, 1957
11 Rössner S., Larsson-Cohn, U Carlson, L. A. & Boberg, J Acta Med Scand 190 301 1971
12. Stokes, T & Wynn, V Lancet II 677 1971
13. Werk, E. E. J & Knowles, H C Jr Diabetes 10- 22, 1961

Submitted fo publication J ne 19 197

Nils-Olov Lunell
Department of Obstetrics and Gynecology
Sabbatsberg Hospital
S-113 82 Stockholm
S eden

tramuscularly. Throughout, the measured concentrations were lower than the calculated ones. The differences are statistically significant. This strongly suggests the occurrence of an inhibitor in the amniotic fluid.

Table II shows the corresponding values of the group where aminoguanidine was not given. No statistically significant difference was found between calculated and measured values of diamine oxidase activity.

In Tables III and IV the concentrations of diamine oxidase activity in amniotic fluid and *pregnant venous plasma are given before and after incubation*. It is obvious that the concentrations before and after incubation are not changed.

DISCUSSION

It is well known that there is a substantial diamine oxidase activity in the amniotic fluid. That this activity is significantly reduced if a diamine oxidase inhibitor (aminoguanidine) is given intramuscularly to the pregnant woman was shown by Tornqvist et al. (4). In the present investigation aminoguanidine has been detected in amniotic fluid with TLC after intramuscular administration.

Table III. *Diamine oxidase concentration in amniotic fluid before and after incubation at 37°C for 1 hour. T some cases aminoguanidine was given before sampling of amniotic fluid*

Case	Aminoguanidine		No aminoguanidine	
	Before incubation (U/l)	After incubation (U/l)	Before incubation (U/l)	After incubation (U/l)
1	0.00	0.49		
2	0.03	0.03		
	0.04	0.03		
4	0.06	0.06		
5	0.02	0.02		
6			0.37	0.35
7			0.22	0.35
8			0.08	0.09
9			0.16	0.14
10			0.03	0.03
11			0.09	0.09
12			0.14	0.16
13			4.87	5.38

The diamine oxidase concentrations given in units per litre and minute.
One unit (U) defined as 1 pmole of substrate degraded per minute at 37°C in sec.

Table IV. *Concentration of diamine oxidase in pregnant venous plasma before and after incubation at 37°C for 1 hour*

Case	Before incubation (U/l)	After incubation (U/l)
1	0.82	0.72
2	0.52	0.45
3	0.77	0.75
4	1.12	1.12
5	0.50	0.59
6	0.59	0.61
7	0.43	0.52
8	0.40	0.58

tion. If amniotic fluid from such women was incubated with pregnant venous plasma from non-treated women the diamine oxidase activity of the mixture was significantly lower than calculated. No corresponding difference was found when amniotic fluid from non-treated women was investigated in the same way. This suggests strongly the presence of a diamine oxidase inhibitor in amniotic fluid after an intramuscular injection of aminoguanidine. Incubation by itself did not change the diamine oxidase activity either of amniotic fluid or venous plasma which excludes the presence of naturally occurring diamine oxidase inhibitors in these compartments. Thus it is highly probable that diamine oxidase inhibition *of amniotic fluid after an intramuscular injection* of aminoguanidine, at least to some extent, must be due to the passage of the inhibitor into the amniotic fluid. In this context the first experiment in Table I is interesting. There is no sign of diamine oxidase inhibition in the amniotic fluid after injection of aminoguanidine although the venous plasma of this woman (figures not given) showed marked enzyme inhibition. At the sampling, the amniotic fluid in this case was discoloured in a way which suggested intra-uterine death of the foetus. The aborted foetus was examined and thus the diagnosis confirmed. Without intact *foetal circulation* the passage of aminoguanidine from the maternal circulation to the amniotic fluid might be impossible.

The results of the present investigation support the modern concept that a vast majority of substances given to the mother passes over to the foetus.

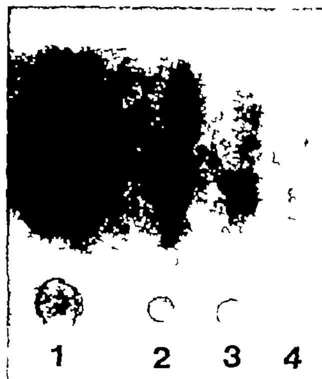


Fig 1 Thin layer chromatography of amniotic fluid after an intramuscular injection of aminoguanidine *1* Amniotic fluid sampled after intramuscular injection of aminoguanidine Amniotic fluid + minoguanidine (1 mg/10 ml). *3* Amniotic fluid + arginine (1 mg/10 ml). *4* Amniotic fluid + creatinine (1 mg/10 ml)

amniotic fluid and venous plasma it was possible to calculate an enzyme concentration in the mixt re By comparing this calculated value and the measured diamine oxidase activity of the compound it was possible to judge if the amniotic fluid had any inhibitory effect on the enzyme The group which received aminoguanidine was then compared with the non-treated group To exclude any effect of the incubation itself on the diamine oxidase activity the initial concentrations of amniotic fluid and of the plasma were compared with the corresponding concentrations after incubation.

Statistics

The Wilcoxon tw sample rank test was used to show statistically significant differences bet een groups.

RESULTS

With TLC definite spots of aminoguanidine were shown in the amniotic fluid after an intra muscular injection of aminoguanidine sulphate to the pregnant women. The spots were best de tected with the PCF reagent. The spots were first blue changing to light red, after which they faded in 5 minutes. Fig. 1 shows a photograph of a

Table I *Calculated and measured diamine oxidase concentrations in a mixture of pregnant venous plasma and amniotic fluid The cases from which amniotic fluid was sampled had previously received aminoguanidine intramuscularly*

Case	Pregnant venous plasma (U)	Amniotic fluid (U)	Calculated value of the mi ture (U/l)	Measured value of the mixture (U/l)
1	0.80	0.40	0 80	0.73
2	0.43	0 02	0.13	0 04
3	0.52	0 03	0 15	0.06
4	0.76	0 04	0.22	0.06
5	0.50	0.06	0 17	0.08
6	0.34	0.1	0 18	0.12
7	0.50	0.06	0.17	0.08
8	0.40	0.03	0.13	0.07
9	0.49	0.0_	0.14	0 07
10	0.37	0.02	0.11	0 03

The diamine oxidase concentration given in units per litre and minute.
One unit is defined as 1 μmole of substrate degraded per minute at 37°C in air

typical experiment with the PCF reagent. The figures make it highly probable that aminoguanidine was present in the amniotic fluid.

Table I shows calculated and measured values of diamine oxidase activity in a 1 to 3 parts mix ture of pregnant venous plasma and amniotic fluid. The cases from which amniotic fluid was sampled all received aminoguanidine sulphate in-

Table II *Calculated and measured diamine oxidase concentrations in a mixture of pregnant venous plasma and amniotic fluid without administration of aminoguanidine*

Case	Pregnant enous plasma (U/l)	Amniotic fluid (U/l)	Calculated value of the mixture (U/l)	Measured alue of the mixture (U/l)
1	0.82	0.37	0 48	0 45
2	0.52	0.22	0 30	0 49
3	0.77	0.08	0 _5	0.25
4	1 12	0 16	0 40	0 40
5	0.59	0 09	0.22	0 25
6	0.89	0 03	0._5	0 25
7	0.55	0.14	0 24	0 26
8	0.35	4.87	3 74	5 18
9	0 66	0.14	0 27	0 30

The diamine oxidase concentration given i unit per litre and minute
One unit (U) def ned as 1 μmole of substrate degraded per mi ute t 37 C in ai

Acta Obstet Gynec Scand 52, 29–36, 1972

THE EFFECT OF NORMAL AND ABNORMAL LABOUR ON THE FOETUS

A Survey

L. S. Persianinov

From the All-Union Research Institute for Obstetrics and Gynaecology of the USSR Ministry of Health (Head: Professor L. S. Persianinov), Moscow, USSR

The state of the foetus during pregnancy can be determined not only by routine clinical examination, but also by electrocardiography, electroencephalography, amnioscopy and investigation of gas exchange and the acid–base balance in the foetal blood.

The literature and the author's own research (6, 7, 8, 9, 10, 11, 12, 13) show that during a normal pregnancy the foetus is in a condition of reduced oxygenation and moderate acidosis.

At the onset of labour the conditions of intrauterine life alter, since there is a rise in intrauterine pressure and compression of the intermuscular vessels of the uterus and of the retrouterine part of the maternal aorta. In addition there are changes in the neurohumoral correlations and metabolic processes in the maternal organism. These changes result in alterations in placental blood pressure and diminished flow of maternal blood to the placenta (1, 3, 6, 19 and others).

Providing compensatory adaptation and normal coordinated uterine myometrial activity occurs during normal labour, these phenomena do not cause pathological hypoxia of the foetus or change its condition.

During the first stage of physiological labour there are no marked changes in the heart action of the foetus and (as needs to be specially stressed) no changes of cardiac rate during uterine contractions. In the second stage, however, there may be a considerable effect on the foetus, due to such factors as compression during its passage through the birth canal, disturbance of uterine placental circulation during expulsive contractions, maternal hypoxia, etc. The foetal reactions in response to these phenomena may manifest themselves in a change in heart rate, which is the best index of the foetal condition. In a normal birth, bradycardia is usually noted in the second stage and is most pronounced when the head passes through the narrow part of the pelvis. The heart beat drops (from 110 to 80 per minute) 10 or 12 seconds after the beginning of an expulsive contraction and returns to normal within 30 seconds of its termination.

In 10% of foetuses tachycardia is observed (up to 185 beats a minute). It is most pronounced at the height of an expulsive contraction and diminishes within 15 seconds of its termination.

These changes in foetal heart action during the second stage of labour do not have any noticeable effect on neonatal respiratory function. Comparison of the respiratory function of foetuses extracted by caesarean section and those delivered after a normal pregnancy and labour shows only a difference in the oxygen saturation of the blood. The values of carbon dioxide partial pressure (pCO_2) and base deficiency (BE) are almost identical.

An abnormal labour and the obstetrical manipulations and operations associated with it, maternal blood loss, and the administration of anaesthesia have a profound effect on the foetus, and this is reflected in its heart action, respiratory function, and metabolism in general. During complicated labour, pathological acidosis, impairment of electrolyte balance, etc. develop irrespective of the nature of the complication.

Foetal hypoxia often occurs in association with

REFERENCES

1 Smith, I Chromatographic and Electrophoretic Techniques, part I pp. 222 230. Heinemann Medical Books Ltd, London, 1960.
2 Sthal, E. Dünnschichtschromatographie pp. 53–76 Springer Verlag, 1967
3 Tryding, N Scand J Clin Lab Invest *17* suppl. *86* 197 1965
4 Törnqvist, A., Jonassen, F Johnson, P & Fredholm, A. M Acta Obstet Gynec Scand *50* 79–84, 1971

Submitted for publication June 20 1972

Ake Törnqvist
Dept of Obstetrics and Gynaecology I
Sahlgrenska sjukhuset
413 45 Gothenburg
Sweden

Acta Obstet Gynec Scand 52, 29–36, 1972

THE EFFECT OF NORMAL AND ABNORMAL LABOUR ON THE FOETUS

A Survey

L. S. Persianinov

From the All-Union Research Institute for Obstetrics and Gynaecology of the USSR Ministry of Health (Head: Professor L. S. Persianinov), Moscow, USSR

The state of the foetus during pregnancy can be determined not only by routine clinical examination, but also by electrocardiography, electroencephalography, amnioscopy and investigation of gas exchange and the acid–base balance in the foetal blood.

The literature and the author's own research (6, 7, 8, 9, 10, 11, 12, 13) show that during a normal pregnancy the foetus is in a condition of reduced oxygenation and moderate acidosis.

At the onset of labour the conditions of intrauterine life alter since there is a rise in intrauterine pressure and compression of the intermuscular vessels of the uterus and of the retro-uterine part of the maternal aorta. In addition there are changes in the neurohumoral correlations and metabolic processes in the maternal organism. These changes result in alterations in placental blood pressure and diminished flow of maternal blood to the placenta (1, 3, 6, 19 and others).

Providing compensatory adaptation and normal coordinated uterine myometrial activity occurs during normal labour, these phenomena do not cause pathological hypoxia of the foetus or change its condition.

During the first stage of physiological labour there are no marked changes in the heart action of the foetus and (as needs to be specially stressed) no changes of cardiac rate during uterine contractions. In the second stage, however, there may be a considerable effect on the foetus, due to such factors as compression during its passage through the birth canal, disturbance of uterine placental circulation during expulsive contractions, maternal hypoxia, etc. The foetal reactions in response to these phenomena may manifest themselves in a change in heart rate which is the best index of the foetal condition. In a normal birth, bradycardia is usually noted in the second stage and is most pronounced when the head passes through the narrow part of the pelvis. The heart beat drops (from 110 to 80 per minute) 10 or 12 seconds after the beginning of an expulsive contraction and returns to normal within 30 seconds of its termination.

In 10% of foetuses tachycardia is observed (up to 185 beats a minute); it is most pronounced at the height of an expulsive contraction and diminishes within 15 seconds of its termination.

These changes in foetal heart action during the second stage of labour do not have any noticeable effect on neonatal respiratory function. Comparison of the respiratory function of foetuses extracted by caesarean section and those delivered after a normal pregnancy and labour shows only a difference in the oxygen saturation of the blood.

The values of carbon dioxide partial pressure (pCO_2) and base deficiency (BE) are almost identical.

An abnormal labour and the obstetrical manipulations and operations associated with it, maternal blood loss, and the administration of anaesthesia have a profound effect on the foetus, and this is reflected in its heart action, respiratory function and metabolism in general. During complicated labour pathological acidosis, impairment of electrolyte balance, etc. develop irrespective of the nature of the complication.

Foetal hypoxia often occurs in association with



Fig. 1. Dynamics of foetal cardiac rhythm during application of forceps with weak uterine contractions. *1* Maternal ECG. *2* Foetal ECG before application of forceps. *3* Foetal ECG during application of forceps. *4* increasing foetal bradycardia during traction.

weak contractions resulting in a protracted labour or by too strong or uncoordinated contractions with short intervals, or spastic contractions of the uterine muscle.

The administration of drugs to stimulate contractions has an unfavourable effect on the foetus only when the uterine contractions become very strong and protracted causing disturbances of uterine placental circulation and consequent hypoxia and acidosis of the foetus. These phenomena are more likely to occur when intravenous injection of oxytocin is given at a rate of 40 or more drops per minute of a solution containing 5 I U oxytocin per 500 ml 5% glucose.

Surgical procedures such as podalic version and breech extraction application of forceps and vacuum extractor cause reflex changes in foetal heart action resembling those associated with hypoxia. This is manifested by irregular intensity and duration of the sounds, by the appearance of individual murmurs, and by impairment of the rate and rhythm of the heart beat. When forceps are used, more pronounced changes in foetal heart action are observed in response to the introduction of the blades (especially in cephalic applications) the changes are greatest during traction and lessen or disappear in the intervening periods. Bradycardia of varying degree occurs characteristically during cephalic traction by means of forceps (Fig. 1). Our own findings (16) and those of Ullery et al. (18) Chachava (?) and others, are that the force and duration of traction, and the intervals between tractive effort, have a bearing on the depression and the restoration of heart rate. During forceps traction, the following changes in foetal ECG are observed, in addition to bradycardia, inversion flattening or a biphasic character of the T wave and displacement of the ST segment from the isoelectric line. During traction by means of a vacuum extractor bradycardia or sinustachycardia are encountered other changes in the ECG are less common than with traction by forceps.

An observation of great importance is that the use of anaesthesia (ether–oxygen and other types) *for obstetric operations per vagina reduces the* number and strength of fetal reflex responses (Fig. 2) and slightly lowers the excitability of its respiratory centre thus reducing the danger of true (extra uterine) respiratory effort developing in the foetus before birth.

The stress upon the foetus is greater when the umbilical cord is coiled around the neck or is prolapsed. These complications lead to impairment of heart action and the development of acidosis.

In prolonged pregnancy the foetus becomes very sensitive to various stimuli and to oxygen deficiency. Changes also occur during rupture of the membranes and palpation of the foetal head, and are manifested as lability of cardiac rhythm and transitory bradycardia, the latter being intensified in the second stage of labour.

The author's experience is that hypoxia and acidosis are observed in all foetuses during prolonged pregnancy with disturbed heart action (bradycardia, changes of configuration and inversion of the T wave lengthening of the electrical systole Hegglin's phenomenon in expulsive contractions, and curving and splitting of the R wave of the QRS complex) (Fig. 3).

The best clinical test for determining the functional state and adaptative capacity of the foetus during labour is the change in foetal heart rate during uterine contractions in the first stage of labour and during vaginal examination (palpation of the presenting part).

In healthy foetuses these reactions are transitory and are not pronounced. When foetal resistance to external stimuli is weakened for various reasons and there are signs of marked hypoxia

Fig. 1. Alteration of the foetal heart rate during breech extraction, following podalic version in transverse presentation. *I*, Without anaesthesia, *II* under anaesthesia. *A*, Heart rate before breech extraction; *B* during extraction; *a*, grip on the foot, *b* delivery of shoulders, extraction of the head. Tachocardiogram made in each cardiac cycle. *Abscissa* time in seconds. *ordinate* heart beats per minute.

or injury the response reactions are more pronounced and are manifested as greater changes of heart action, they usually precede intra-uterine asphyxia and are a warning of it.

Complicated labour has most unfavourable effect on premature babies, whose adaptational mechanisms are not strong enough to combat oxygen deficiency. Hypoxia supervenes, and the impaired haemodynamics and weakness of the vascular walls contribute to intracranial haemor-

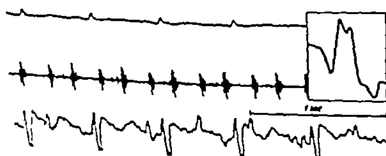


Fig. 2. Marked foetal ECG changes in prolonged pregnancy. Top to bottom: maternal ECG, foetal PCG, foetal ECG, foetal heart complex (enlarged).



Fig. 1. Dynamics of foetal cardiac rhythm during application of forceps with weak uterine contractions. *1* Maternal ECG. *2* Foetal ECG before application of forceps, *3* Foetal ECG during application of forceps. *4* Increasing foetal bradycardia during traction.

weak contractions resulting in a protracted labour or by too strong or uncoordinated contractions with short intervals, or spastic contractions of the uterine muscle

The administration of drugs to stimulate contractions has an unfavourable effect on the foetus only when the uterine contractions become very strong and protracted causing disturbances of uterine placental circulation and consequent hypoxia and acidosis of the foetus. These phenomena are more likely to occur when intravenous injection of oxytocin is given at a rate of 40 or more drops per minute of a solution containing 5 I U oxytocin per 500 ml 5% glucose

Surgical procedures such as podalic version and breech extraction application of forceps and vacuum extractor cause reflex changes in foetal heart action resembling those associated with hypoxia. This is manifested by irregular intensity and duration of the sounds, by the appearance of individual murmurs, and by impairment of the rate and rhythm of the heart beat. When forceps are used, more pronounced changes in foetal heart action are observed in response to the introduction of the blades (especially in cephalic applications) the changes are greatest during traction and lessen or disappear in the intervening periods. Bradycardia of varying degree occurs characteristically during cephalic traction by means of forceps (Fig. 1). Our own findings (16) and those of Ullery et al (18) Chachava (2) and others, are that the force and duration of traction and the intervals between tractive effort, have a bearing on the depression and the restoration of heart rate. During forceps traction the following changes in foetal ECG are observed, in addition to bradycardia. inversion flattening or a biphasic character of the T wave and displacement of the ST segment from the isoelectric line During traction by means of a vacuum extractor bradycardia or sinustachycardia are encountered other changes in the ECG are less common than with traction by forceps.

An observation of great importance is that the use of *anaesthesia (ether–oxygen and other types)* for obstetric operations per vagina reduces the number and strength of fetal reflex responses (Fig 2) and slightly lowers the excitability of its respiratory centre thus reducing the danger of true (extra uterine) respiratory effort developing in the foetus before birth.

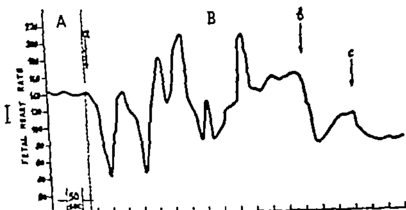
The stress upon the foetus is greater when the umbilical cord is coiled around the neck, or is prolapsed These complications lead to impairment of heart action and the development of acidosis.

In prolonged pregnancy the foetus becomes very sensitive to various stimuli and to oxygen deficiency Changes also occur during rupture of the membranes and palpation of the foetal head, and are manifested as lability of cardiac rhythm and transitory bradycardia the latter being intensified in the second stage of labour

The author's experience is that hypoxia and acidosis are observed in all foetuses during prolonged pregnancy with disturbed heart action (bradycardia, changes of configuration and inversion of the T wave lengthening of the electrical systole Hegglin s phenomenon in expulsive contractions, and curving and splitting of the R wave of the QRS complex) (Fig. 3)

The best clinical test for determining the functional state and adaptative capacity of the foetus during labour is the change in foetal heart rate during uterine contractions in the first stage of labour and during vaginal examination (palpation of the presenting part)

In healthy foetuses these reactions are transitory and are not pronounced. When foetal resistance to external stimuli is weakened for various reasons and there are signs of marked hypoxia



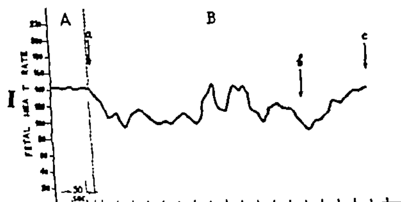


Fig. 2. Alteration of the foetal heart rate during breech extraction, following podalic version in transverse presentation. *I*, Without anaesthesia, *II*, under anaesthesia. *A*, Heart rate before breech extraction; *B*, during extraction; *a*, grip on the foot; *b*, delivery of shoulders; *c*, extraction of the head. Tachocardiogram made in each cardiac cycle. *Abscissa*: time in seconds, *ordinate*: heart beats per minute.

or injury the response reactions are more pronounced and are manifested as greater changes of heart action; they usually precede intra-uterine asphyxia and are a warning of it.

Complicated labour has a most unfavourable effect on premature babies, whose adaptational mechanisms are not strong enough to combat oxygen deficiency. Hypoxia supervenes, and the impaired haemodynamics and weakness of the vascular walls contribute to intracranial haemor-

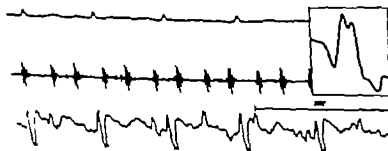


Fig. 3. Marked foetal ECG changes in prolonged pregnancy. Top to bottom: maternal ECG, foetal PCG, foetal ECG, foetal heart complex (enlarged).

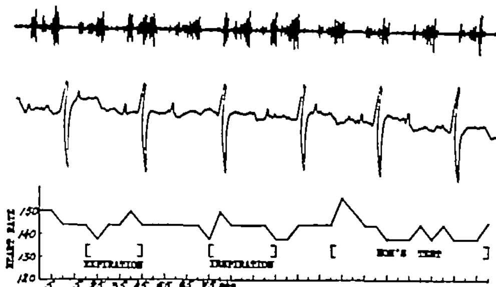


Fig 4 Changes of foetal heart action with coiling of the umbilical cord around the neck. Top to bottom: Foetal PCG, ECG and tachocardiogram. There are changes in volume and the appearance of murmurs on the PCG.

rhage, which further worsens the foetal condition. The incidence of intracranial haemorrhage in premature babies is several times that in full-term ones.

Our own numerous studies of pregnancy point to a possibility of determining foetal tolerance which is of great practical importance.

Several pathological conditions (toxaemia, prolonged pregnancy diabetes, Rh-incompatibility etc.) are accompanied by impairment of placental function which disturbs uterine-placental circulation and causes chronic hypoxia of the foetus. Yet owing to compensatory mechanisms no noticeable variations arise in the acid–base balance *of the foetal blood and no spontaneous changes* in the phono- and electrocardiograms. Functional tests on pregnant women (holding the breath thermal stimulation of the abdominal skin, mild physical exercise) that change the intra-uterine conditions of the foetus are manifested in the weakening of the heart action to the point of its complete cessation, and distortion which sometimes acquires a *paradoxical character*

In conditions of chronic hypoxia childbirth especially a complicated delivery easily leads to exhaustion of the foetus reserves, the development of acute hypoxia and increased metabolic acidosis.

Coiling of the cord around the neck or other parts of the foetus is encountered in 15 to 30 of all deliveries. *The ECG changes in this com*plication are basically a constant and marked alteration of the amplitude and duration of the sounds and the appearance of systolic murmurs (Fig. 4 5) Moderate changes of heart rate are encountered only during increased movement of the foetus. When in Rh-incompatibility of mother and foetus there is marked anaemia, with a haemoglobin concentration below 100 %, a systolic murmur is evident on the phonocardiogram

In the ictero-anaemic form of haemolytic disease a moderate reduction of sound amplitude and of the foetal QRS complex are seen. In cases of hydrops foetalis there is a sharp reduction in the sound amplitude and of the QRS complex. In some cases splitting or flattening of the peak of the R wave is encountered.

Compression of the maternal inferior vena cava *which often occurs toward the end of pregnancy* causing changed haemodynamics in the mother provokes an accelerated foetal heart rate. When the mother's systolic blood pressure falls by 15 to 20 mmHg below its initial level, the foetal pulse rate gradually increases by 4–4 per minute, with a subsequent prolonged stabilization at the elevated level.

A gradual decrease of foetal heart rate by 60 or more per minute is observed when the mother's systolic pressure drops by 30 mmHg or more or when there is vascular collapse Foetal heart action becomes normal 35 to 50 seconds after the mother is turned onto her side (Fig 6)

It is very important to diagnose chronic foetal hypoxia early no matter what its cause (pathology *of the placenta of the umbilical cord, etc.*) Its most demonstrative sign on the PCG is monotony of rhythm the cardiotachogram often having the form of a straight line (Fig. 7)

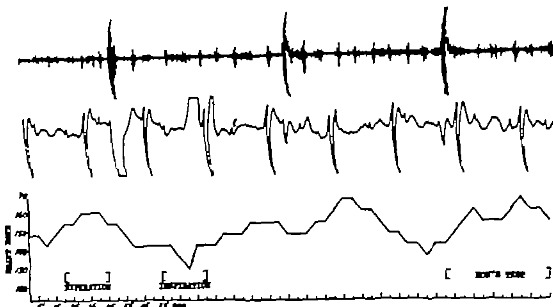


Fig. 5. Changes of foetal heart action with coiling of the umbilical cord around the neck and extremities. The changes in volume, appearance of murmurs, and individual oscillations of the PCG indicate tightening of the cord about the extremities.

If the monotony of rhythm is associated with considerable reduction in the QRS complex, or a gradual decrease of amplitude in continuous recording, there is no doubt that chronic asphyxia *threatens the life of the foetus and that urgent* measures are called for.

Unlike dangerous asphyxia, early asphyxia of the foetus is characterized by a significant irregularity of the heart beat manifested by alternation of slow and rapid phases. When signs of chronic hypoxia and reflex changes in foetal heart action appear during pregnancy and labour Nikolaev's triad is valuable (intravenous injection of 50 ml of 40% glucose with 300 mg of ascorbic acid, *cardiozol, and oxygen inhalation*). Intravenous (1 ml of 2% Sigetin) and intramuscular (oestradiol dipropionate) injections of oestrogen preparations are effective (Fig. 8)

Dipotassium [illegible]-3,4 diphenyl-[illegible].



Fig. 6. Changes in the foetal heart rate in syndrome of compression of the inferior vena cava. Above tachocardiogram, below phonocardiogram.

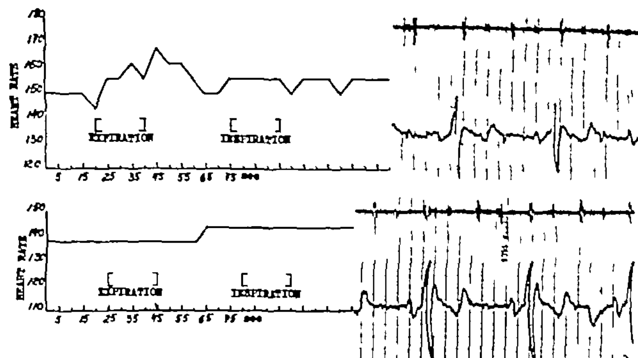
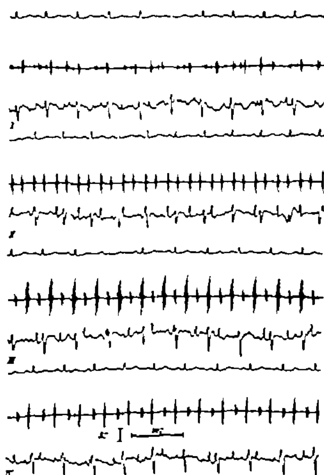


Fig. 7. Foetal heart action in dynamics in chronic hypoxia. *I*, 38th week of pregnancy: ECG and PCG normal. *II*, 39th week, diminishing amplitude of the QRS complex on the ECG and monotony of rhythm on the cardiotachogram 14 hours bef re foetal death.



The close relation between the quantity of acid products and alkaline buffer substances in the foetal and maternal blood (12) allows the impaired respiratory function of the foetus to be restored via the maternal system (14, 16). In the presence of acidosis in the mother (diabetes, prolonged pregnancy, prolonged labour, severe toxaemia, etc.) and in disturbances of foetal heart action, it is expedient to give the mother an intravenous injection of 150 to 200 ml of 5% sodium bicarbonate followed by 100 ml of 10 to 20% glucose (Fig. 9).

Determination of foetal tolerance during pregnancy reveals how seriously the approaching birth will threaten it. Detection of the initial symptoms of foetal distress in some cases enables them to be eliminated by therapeutic and prophylactic measures. This may render possible a normal vaginal delivery with a favourable outcome for both mother and baby. In other cases increasing

Fig. 8. Changes of foetal heart action in the onset of asphyxia during labour: the mother is given ml of a solution of Sigetin together with 20 ml of a 40% solution of glucose; top to bottom: Maternal ECG, foetal PCG, foetal ECG. *I*—before injection of Sigetin, 15 (*II*), 30 (*III*) and 120 minutes (*IV*) after injection.

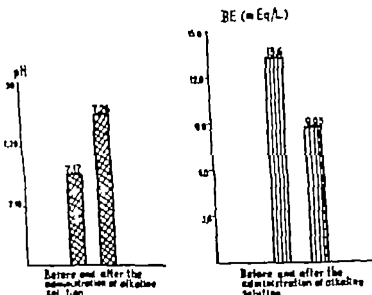


Fig. 9. Decrease of acidosis in the foetus shown by changes of pH and BE values after injection of alkaline solution into the mother.

distress of the foetus revealed by repeated examination calls for careful premature delivery with simultaneous application of measures to improve the foetal state by normalizing its heart action and acid–base balance.

The choice of method and time of delivery depend on the state of the foetus and the obstetrical situation but in fetal distress the delivery should be as easy as possible for the baby.

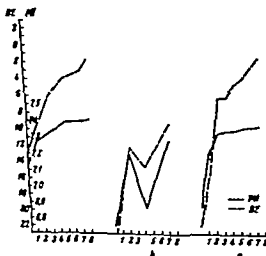


Fig. 10. Blood pH and BE indices in normal birth (*a*), in asphyxia (*b*), and after alkali and glucose injection in severe asphyxia (*c*).

When a baby is born in a state of asphyxia the resuscitative measures should include injection of an alkaline solution into the umbilical vein (15, 4, 9 and others).

Since 1965 we have been using 10 to 20 ml of 5% sodium bicarbonate (in severe asphyxia 15 to 25 ml according to the foetal weight) with subsequent injection into the umbilical vein of 10% glucose (8 to 10 ml per kg of the foetal weight) and carboxilase (8 mg per kg of the foetal weight). In cases of secondary asphyxia or of pathological metabolic acidosis in the first days of life it is necessary to resort to infusion of 5% sodium bicarbonate. The widespread use of alkaline solutions during pregnancy and labour in the above mentioned conditions and in asphyxia of the new born (Fig. 10) made it possible to reduce the neonatal death-rate of the newborn in the USSR by 50% (9) and considerably diminish the number of complications of various kinds.

REFERENCES

1. Caldeyro-Barcia, R. & Poseiro, J. J. Fetal and maternal dangers due to misuse of oxytocin. Montreal, 1958.
2. Chacheva, K. V. A new method of obtaining an electrocardiogram of the fetus. Tbilisi, USSR, 1965.
3. Hon, E. H. & Hess, O. W. The electronic evaluation of foetal heart rate. Amer J Obstet Gynec 82, 1055, 1961.
4. James, L. S. Physiology of respiration in newborn in-

fants and in the respiratory distress syndrome. Pediatrics *24* 1069 1959
5 Kelly J V Amer J Obstet Gynec *87* 529 1963.
6. *Persianinov L. S. & Cherwakova, T V., Influence* of obstetrical manipulation and operative interventions on the heart activity of the fetus. Vestnic Akad Med Nauk *11* 1962.
7 Persianinov L. S The influence of labour on the foetus. J Intern Fed Gynec Obstet *4* 1966
8 — Pathological metabolic acidosis in foetal disorders. Proceedings of a Symposium, Prague, 1966. *In* Intra-uterine Dangers to the Foetus. Excerpta Medica Foundation, p. 411 1967
9 — Asphyxia in the Foetus and Newborn. Medicina, Moscow 1961 1967
10. — Effect of abnormal labour on the foetus. Fifth World Congress of Gynaec and Obstetr Australia, 1967
11 Persianinov L. S. et al. Foundation Clinical cardiology of the Foetus, *Medicina, Moscow 1967 Warsaw* 1968
12. Persianinov L. S. & Savelieva, G M Disturbances in the respiratory functions of foetal blood and their treatment. J Intern Feder Gynec Obstet *6*, 4 1968.
13. Persianinov L. S. Asfixia del feto y del recién nacido. Editorial Mir Moscow 1970.
14 Rooth, G., Early detection and prevention of foetal acidosis. *Lancet I 7328 290, 1964.*
15 Saling, E.: Das Kind im Bereich der Geburtshilfe. Stuttgart, 1966.
16. Saveleva, G M Normal gas composition and acid-base balance in the fetal and maternal blood. Obstet Gynec (Moscow) *6*. 33 1966.
17 Steinbera, Z. k. et al. Complex diagnosis of intrauterine foetal distress. *In* Intra-uterine Dangers to the Foetus. Excerpta Medica Foundation, p. 373 1967
18 Ullery J G Amer J Obstet Gynec *85* 1963.
19 Vasicka, A. & Hutchinson, H. Does uterine contractility cause foetal bradycardia. J Obstet Gynec *1*. 409 1963

Submitted for publication June 20 1972

L. S. Persianinov
The All-Union Institute
of Obstetrics and Gynaecology
Elanskogo 2
Moscow G-435
USSR

Acta Obstet Gynec Scand 52: 37–40, 1973

PLASMA LEVELS OF PROGESTERONE AND OESTRADIOL AFTER INJECTIONS OF 17α-HYDROXYPROGESTERONE CAPROATE DURING THE LUTEAL PHASE OF THE NORMAL MENSTRUAL CYCLE

Elof D. B. Johansson

From the Department of Obstetrics and Gynaecology (Head: Professor Carl Gemzell), University Hospital, Uppsala, Sweden

Abstract. 17α-hydroxyprogesterone caproate (Proluton Depot®, Schering AG, Berlin) was given after ovulation in a dose of 500 mg intramuscularly to four women with normal menstrual cycles. The plasma levels of progesterone and oestradiol were measured and compared to the levels found during previous control cycles of the same women. In the four normal women a small reduction of the plasma levels of progesterone and oestradiol was found after the injection of 500 mg of 17α-hydroxyprogesterone caproate. However the number of days with progesterone concentrations above the mean level of the follicular phase was not changed. The menstrual bleeding was delayed and appeared to be a withdrawal bleeding from 17α-hydroxyprogesterone caproate rather than from progesterone.

In previous reports from this laboratory it has been shown that synthetic gestagens can cause depression of progesterone and oestrogen synthesis during the luteal phase of the normal menstrual cycle (7, 9, 10). The 19-nortestosterone derivatives were found to be more potent in inhibiting progesterone and oestradiol synthesis in the corpus luteum than derivatives of acetoxyprogesterone. A total dose of 300 mg or more of chlormadinone acetate or medroxyprogesterone was required to produce a significant decrease in the plasma levels of progesterone while significant effects could be achieved with much smaller doses of the 19-nortestosterone derivatives. During the early part of pregnancy neither the 19-nortestosterone derivatives or the acetoxyprogesterone derivatives appeared to have any significant effect on steroidogenesis as judged by the plasma levels of progesterone and oestradiol (14). As 17α-hydroxyprogesterone caproate is widely used clinically as a gestagen during the menstrual cycle and pregnancy it was of special interest to study the effect of this compound on the function of the corpus luteum during the normal menstrual cycle.

MATERIALS AND METHODS

Four young and healthy women, free of gynaecological disorders, volunteered in this study. These women were accustomed to venopunctures during previous studies and their pattern of oestrogen and progesterone during several normal cycles was well known (6, 9, 10). Collection of daily venous blood samples was started during the follicular phase well before the rise of oestrogen during one control cycle and one treated cycle in each woman. A single intramuscular injection of 500 mg of 17α-hydroxyprogesterone caproate (Proluton Depot®, Schering AG, Berlin) was given on the third, fourth or fifth day after the calculated day of ovulation. The day of ovulation was calculated to have occurred on the second day of elevated levels of plasma progesterone coinciding with the marked drop in the plasma levels of oestradiol (11).

Assay methods

The plasma levels of progesterone were measured by the rapid competitive protein binding technique described by Johansson (6, 9). As 17α-hydroxyprogesterone caproate will bind to transcortin used in the progesterone method (Johansson unpublished) parallel determinations were done in a series of samples after separation of progesterone from 17α-hydroxyprogesterone caproate on Sephadex LH-20 columns. No significant difference was found between the samples measured before column-separation as compared to the progesterone levels found after separation on the Sephadex columns.

The plasma levels of oestradiol were measured by radioimmuno-assay technique according to the method described by Hotchkiss et al. (5) with some modifications (2). The antiserum was prepared by Férin et al. (3).

fants and in the respiratory distress syndrome. Pediatrics 24 1069 1959
5 Kelly J V Amer J Obstet Gynec 87 529 1963
6. Persianinov L. S. & Cherwakova, T V.. Influence of obstetrical manipulation and operative interventions on the heart activity of the fetus. Vestnic Akad Med Nauk 11 1962.
7 Persianinov L. S.. The influence of labour on the foetus. J Intern Fed Gynec Obstet 4 1966.
8. — Pathological metabolic acidosis in foetal disorders. Proceedings of a Symposium, Prague, 1966. In Intra-uterine Dangers to the Foetus. Excerpta Medica Foundation, p. 411 1967
9 — Asphyxia in the Foetus and Newborn. Medicina, Moscow 1961 1967
10. — Effect of abnormal labour on the foetus. Fifth World Congress of Gynaec and Obstetr Australia, 1967
11 Persianinov L. S. et al. Foundation Clinical cardiology of the Foetus, Medicina, Moscow 1967 Warsaw 1968
12. Persianinov L. S. & Savelieva, G M Disturbances in the respiratory functions of foetal blood and their treatment. J Intern Feder Gynec Obstet 6. 4 1968.
13 Persianinov L. S.. Asfixia del feto y del recién nacido. Editorial Mir Moscow 1970.
14 Rooth, G Early detection and prevention of foetal acidosis. Lancet I 73..., 290, 1964
15 Saling, E.. Das Kind im Bereich der Geburtshilfe. Stuttgart, 1966.
16. Savelieva, G. M.. Normal gas composition and acid-base balance in the fetal and maternal blood. Obstet Gynec (Moscow) 6 33, 1966.
17 Steinbera, Z. K. et al. Complex diagnosis of intra-uterine foetal distress. In Intra-uterine Dangers to the Foetus. Excerpta Medica Foundation, p. 373, 1967
18. Ullery J G Amer J Obstet Gynec 85, 1963.
19 Vasicka, A. & Hutchinson, H.. Does uterine contractility cause foetal bradycardia. J Obstet Gynec ..2: 409 1963

Submitted for publication June 20 1972

L. S. Persianinov
The All-Union Institute
of Obstetrics and Gynaecology
Elanskogo 2
Moscow G-435
USSR

This woman has never had any cycle longer than 32 days during the last 4 years.

Case IV. IM, 29 years old, has been taking part in our program for 3 years and always shown reproducible control cycles. The injection of 500 mg Proluton Depot® was given on the fifth day after the calculated day of ovulation and was followed by a significant decrease in the plasma levels of progesterone as compared to the control cycle and a slight decrease in the levels of oestradiol. The menstrual bleeding was delayed by 3 days. The next cycle was normal.

The plasma levels of progesterone and oestradiol in the four treated women have been summarized in Fig. 3, and compared to a summation of their respective control cycles. The cycles were synchronized on the calculated day of ovulation. Both the plasma levels of progesterone and oestradiol showed similar levels and patterns in the control and treated cycles up to the period of treatment. During and after the injection period the plasma levels of progesterone were lower than found during the control cycles. A slight decrease was also found in oestradiol levels. However both oestradiol and progesterone levels were elevated above the normal follicular phase levels during the same length of time as in the control cycles. Despite low plasma levels of progesterone and oestradiol the onset of the menstrual bleeding was delayed by several days in the treated cycles. The menstrual bleeding started on the eleventh (? cycles) and twelfth (2 cycles) day after the injection of Proluton Depot®.

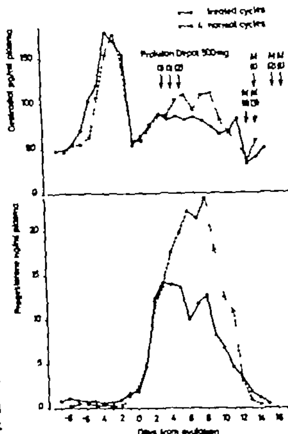


Fig. 3. A composite graph of the plasma levels of progesterone and oestradiol during four control cycles and four cycles treated with an intramuscular injection of 500 mg of 17α-hydroxyprogesterone caproate in four normal women. The cycles are synchronized on the calculated day of ovulation. *M* indicates the first day of the menstrual bleeding in the individual cycles. The points show the mean levels.

DISCUSSION

In a previous report it was shown that the luteal phase could be shortened by 19-nortestosterone derivatives given after ovulation. This shortening of the luteal phase was shown both in the plasma levels of progesterone and on the time of onset of menstrual bleeding (9, 10). Despite the high dose of 17α-hydroxyprogesterone (500 mg) given in this study the plasma levels of progesterone remained elevated above the follicular phase levels for the normal length of the luteal phase. The menstrual bleeding that followed after the injection of 17α-hydroxyprogesterone caproate was a withdrawal bleeding from the drug as the plasma level of progesterone was below 1 ng per ml plasma for several days proceeding the bleeding. In the normal menstrual cycle the bleeding usually started within a day when the plasma levels have fallen below 1 ng per ml (6, 9, 10).

The injections were given at a time when the corpus luteum is considered to be well developed as a gland (1) in order to avoid interference with the formation of the corpus luteum.

As the biological halflife of progesterone is short (4) and the storage in the gland is minimal (15) the peripheral plasma levels of progesterone appeared to be a reliable reflection of the production of progesterone in the corpus luteum. The same conclusion was reached during studies of the metabolic clearance rate of progesterone in the menstrual cycle (13).

Studies of the effects of 17α-hydroxyprogesterone caproate when given to women with short luteal phases is under way. However these studies

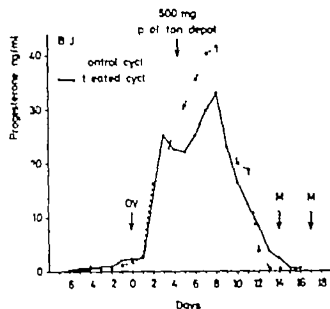


Fig 1 Plasma levels of progesterone during one control cycle and one cycle treated with an intramuscular injection of 500 mg of 17α-hydroxyprogesterone caproate as indicated by the arrow. The cycles are synchronized on the calculated day of ovulation. *M* indicates the first day of vaginal bleeding (Case I).

RESULTS

The effect of a single intramuscular injection of 500 mg 17α-hydroxyprogesterone caproate on the plasma levels of oestradiol and progesterone in the four women studied will be described individually in order to illustrate the individual variations.

Case I BJ 23 years old. This woman has been taking part in our study program for years. Her menstrual cycles ha e varied in length between 8 to 33 days. The plasma levels of progesterone during her luteal phase have always been found to be rather high. 500 mg of Proluton Depot® was given on the fourth day fter the calculated day of ovulation. The difference between the plasma levels of progesterone during the treated cycle as compared to a previous control cycle is shown in Fig. 1. Only a very minor decrease of the plasma levels of progesteron. was seen immediately after the injection. Th. progesterone levels remained elevated during the same number of days as was normal for her luteal phase but the onset of the menstrual bleeding was delayed 3 days as compared to th control cycle despite follicular phase levels of progesterone. The plasma levels of oestradiol during the luteal phase were within the range of the control cycle. The following cycle wa normal in length and was also ovulatory.

Case II GK. 29 years old, has been taking part in our studies f r th last 3 years. Her normal menstrual cycles ha always been found t hav very small variation in the levels of th se steroids. The injection of 500 mg f Proluton Depot® was gi en on the fifth day after the calculated day of ovulation. The oestradiol and progesterone levels found in plasma during the treated cycle and during the previous control cycle are shown in Fig. _. A significant decrease of the plasma levels of progesterone was found but the levels stayed above follicular phase levels for the normal length of time f r this woman. The plasma levels of oestradiol were slightly lower than that of the control cycle but this was true also for the levels before the injection. The start of the menstrual bleeding was delayed by 4 days despite low levels of progesterone. The menstrual cycle that follo ed after the treated cycle was ovulatory and normal in length.

Case III CD. 29 years old, has been taking part in our program for 4 years and always shown reproducible control cycles. The injection was given on the third day after the calculated day of ovulation. A decrease of the plasma levels of progesterone was seen similar to that illustrated in Fig. _, but stayed above follicular phase levels f r the normal length of the luteal phase. A small decrease in plasma oestradiol levels wa also seen. The menstrual bleeding was delayed by only one day as compared to the control cycle. The cycle that followed the treatment cycle lasted for 46 days but was ovulatory

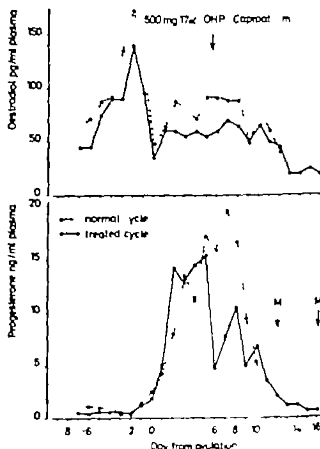


Fig 2 Plasma lev ls of progesterone and oestradiol during one control cycle and one treated cycle w h 500 mg of 17α-hydroxyprogesterone caproa gi en ramuscularly as indicated by the arrow. The cycles re nchronized on the day of vulation. *M* ndicates the first day of the menstrual bleeding (Case II).

This woman has never had any cycle longer than 37 days during the last 4 years.

Case IV. I.M., 29 years old, has been taking part in our program for 3 years and always shown reproducible control cycles. The injection of 500 mg Proluton Depot® was given on the fifth day after the calculated day of ovulation and was followed by a significant decrease in the plasma levels of progesterone as compared to the control cycle and a slight decrease in the levels of oestradiol. The menstrual bleeding was delayed by 3 days. The next cycle was normal.

The plasma levels of progesterone and oestradiol in the four treated women have been summarized in Fig. 3 and compared to a summation of their respective control cycles. The cycles were synchronized on the calculated day of ovulation. Both the plasma levels of progesterone and oestradiol showed similar levels and patterns in the control and treated cycles up to the period of treatment. During and after the injection period the plasma levels of progesterone were lower than found during the control cycles. A slight decrease was also found in oestradiol levels. However, both oestradiol and progesterone levels were elevated above the normal follicular phase levels during the same length of time as in the control cycles. Despite low plasma levels of progesterone and oestradiol the onset of the menstrual bleeding was delayed by several days in the treated cycles. The menstrual bleeding started on the eleventh (2 cycles) and twelfth (2 cycles) day after the injection of Proluton Depot®.

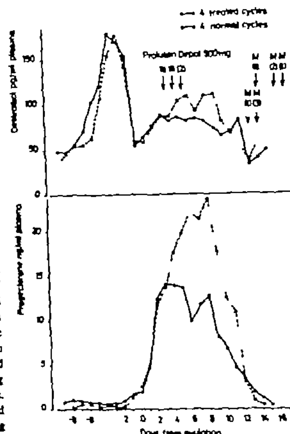


Fig. 3. A composite graph of the plasma levels of progesterone and oestradiol during four control cycles and four cycles treated with an intramuscular injection of 500 mg of 17α-hydroxyprogesterone caproate in four normal women. The cycles are synchronized on the calculated day of ovulation. M indicates the first day of the menstrual bleeding in the individual cycles. The points show the mean levels.

DISCUSSION

In a previous report it was shown that the luteal phase could be shortened by 19-nortestosterone derivatives given after ovulation. This shortening of the luteal phase was shown both in the plasma levels of progesterone and on the time of onset of menstrual bleeding (9, 10). Despite the high dose of 17α-hydroxyprogesterone (500 mg) given in this study the plasma levels of progesterone remained elevated above the follicular phase levels for the normal length of the luteal phase. The menstrual bleeding that followed after the injection of 17α-hydroxyprogesterone caproate was a withdrawal bleeding from the drug as the plasma level of progesterone was below 1 ng per ml plasma for several days proceeding the bleeding. In the normal menstrual cycle the bleeding usually started within a day when the plasma levels have fallen below 1 ng per ml (6, 9, 10).

The injections were given at a time when the corpus luteum is considered to be well developed as a gland (1) in order to avoid interference with the formation of the corpus luteum.

As the biological halflife of progesterone is short (4) and the storage in the gland is minimal (15) the peripheral plasma levels of progesterone appeared to be a reliable reflection of the production of progesterone in the corpus luteum. The same conclusion was reached during studies of the metabolic clearance rate of progesterone in the menstrual cycle (13).

Studies of the effects of 17α-hydroxyprogesterone caproate when given to women with short luteal phases is under way. However, these studies

are complicated by occasional normal cycles in these women. A large number of cycles is therefore required before reliable conclusions can be drawn from the treatment of these abnormal cycles.

During the first trimester of pregnancy the plasma levels of progesterone and oestradiol following an injection of 500 mg of Proluton Depot have only been studied in two patients so far No changes in the plasma levels of progesterone or oestradiol were found, which would indicate that the patients have received a real increase in progestogen active substances by the injection as the effect on target organs is likely to be a summation of the endogenous levels and the amount injected.

It can be concluded from this study that 17α-hydroxyprogesterone caproate in a single intramuscular injection of 500 mg does not shorten the lifespan of the corpus luteum as has been found for 19-nortestosterone derivatives.

ACKNOWLEDGMENT

This study was supported by the Ford Foundation and the Population Council, New York, N Y and Schering AG, Berlin. The author is indebted to the devoted volunteers who made this study possible.

REFERENCES

1 Corner G W., Jr The histological dating of the human corpus luteum of menstruation. Amer J Anat 58 377 1936.
2. Edqvist, L. E. & Johansson, E. D B. Radioimmunoassay of oestrone and oestradiol in human and bovine peripheral plasma. Acta Endocr (Kbh.) 1972, in press.
3 Férin, M Zimmering, P E., Liebermann, S. & Vande Wiele, R. L. Inactivation of the biological effects of exogenous and endogenous estrogens by antibodies to 17β-estradiol. Endocrinology 83 565 1968
4 Fylling, P., Disappearance rate of progesterone following simultaneous removal of the corpus luteum and the foetoplacental unit in women. Acta Endocr (Kbh.) 65 284 1970.
5 Hotchkiss, J Atkinson, L. E. & Knobil, E. Time course of serum estrogen and luteinizing hormone (LH) concentrations during the menstrual cycle of the rhesus monkey Endocrinology 89 177 1971.
6. Johansson, E. D. B., Progesterone levels in peripheral plasma during the luteal phase of the normal menstrual cycle measured by a rapid competitive protein binding technique Acta Endocr (Kbh.) 61 592 1969
7 — A simplified procedure for the assay of progesterone. Karolinska Symposia on Research Methods in Reproductive Endocrinology 2nd Symposium. Acta Endocr (Kbh.), Suppl. 147 188, 1970.
8. — Luteolytic effects of gestagens. Third International Congress on Hormonal Steroids, Hamburg, Germany Excerpta Medica Int. Congress Series No. 10, Abstract 459 p. 15
9 — Luteolytic effect of gestagens. Acta Obstet Gynec Scand 50 75 1971.
10. — Depression of the progesterone levels in plasma in women treated with synthetic gestagens after ovulation. Acta Endocr (Kbh.) 68. 779 1971
11 Johansson, E. D. B. & Wide, L. Periovulatory levels of plasma progesterone and luteinizing hormone in women. Acta Endocr 62 82, 1969
12. Johansson, E. D B Wide, L. & Gemzell, C. Luteinizing hormone (LH) and progesterone in plasma and LH and oestrogens in urine during 4 normal menstrual cycles. Acta Endocr (Kbh.) 68 502, 1971
13 Little B., Lin, T J & Billiar R. B. The metabolic clearance rate of progesterone in the menstrual cycle and per cent interconversion to 20α-dihydroprogesterone Excerpta Med (Amst.) Int rn Congr Series No. 210 211 1970.
14 Nygren, K.-G. & Johansson, E. D B. Interference with the normal function of the corpus luteum by oral administration of synthetic gestagens. VIII Acta Endocr Congress, July 4–8 1971 Copenhagen. Acta Endocr (Kbh.), Suppl. 155 170, 1971 (abstract only).
15 Zander J Forbes, T R., von Münstermann, A. M. & Neher R. Δ 4-3-Ketopregnene-20α-ol and Δ 4-3-Ketopregnene-20β-ol, two naturally occurring metabolites of progesterone Isolation, identification, biologic activity and concentration in human tissues. J Clin Endocr 18 337 1958

Submitted for publication June 30 197

Elof D B. Johansson
Department of Obstetrics and Gynaecology
University Hospital
S-750 14 Uppsala 14
Sweden

Acta Obstet Gynec Scand 52 41–46, 1973

EQUIPMENT FOR DIRECT FHR MONITORING DURING LABOUR

A Comparative Artefact Study

B Westin and G Söderberg

From the Department of Obstetrics and Gynaecology (Head: Professor H. Johansson), Danderyds Hospital, Danderyd, and the Department of Obstetrics and Gynaecology (Head: Professor A. Ingelman-Sundberg), Sabbatsberg Hospital, Stockholm, Sweden

Abstract. Electronic FHR-monitoring is, like monitoring of the heart in adults, combined with artefacts. In the fetus interpretation of artefacts and diagnosis of cardiac disease are facilitated, if the filtered R-wave is presented simultaneously with FHR. Changes in rate depending on artefacts can be misinterpreted with commercial instruments as depending on "fetal distress" which can be avoided with the equipment described in this paper. Filter properties and further reduction of noise are discussed.

Electronic FHR monitoring during delivery has not until recently been transferred from research to clinical practice. Important contributions in this field have been made by Hon (3 and 6), Caldeyro-Barcia (1) and Hammacher (2).

The recent development of relatively simple to-operate instruments for routine obstetrical use present fetal heart rate with slow paper speed. On the one hand such slow paper speed makes it easier to interpret FHR-decelerations but on the other hand makes the interpretation of artefacts difficult. Reliable interpretation of the variations of frequency tracing cannot be performed without simultaneous recording of the trigger signal. Although efforts have been made to reduce noise in FHR-monitoring (4) discrimination in the input stage of the succeeding amplifier and filter properties may probably be improved. Attempts have therefore been made to develop an instrument that satisfies some of the criteria here presented. Comparison will be made between the equipment built by the present authors and some commercially available instruments.

TEST EQUIPMENT

Scalp electrode. At high stations and when the cervical canal was narrow the electrode used was a modified clip probe as described elsewhere (10 and 9). At other stations of the fetal head commercially available noise reducing bipolar silver-silverchloride scalp electrodes described by Hon (5) were used.

Fetal electrocardiographic signal (FECG)-amplifier and band-pass filter. Because of the high discrimination in the input stage of the succeeding amplifier we have been able to eliminate a suppressing electrode which is not customary with commercially available equipment. The FECG-signal obtained from the fetus is connected to the input of an amplifier with high discrimination and is amplified to the output level of 1 V in order to be filtered later in a narrow band-pass filter with a mean frequency of 28 Hz and steep flanks (about 30 and 12 dB per octave, respectively) (Figs. 1 and 2).

To get necessary signal level in order to drive the oscilloscope and the recorder the signal that emanates from the FECG-amplifier has been charged the input of an amplifier of standard model (EMT 12 B Elema Schönander AB)

Heart ratemeter

This custom built instrument (EMT 50 Elema Schönander AB) consists of two units; an amplification unit and

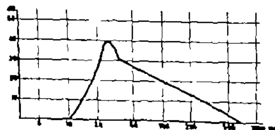


Fig. 1. Band pass filter. Centre frequency 28 Hz.

are complicated by occasional normal cycles in these women A large number of cycles is therefore required before reliable conclusions can be drawn from the treatment of these abnormal cycles.

During the first trimester of pregnancy the plasma levels of progesterone and oestradiol following an injection of 500 mg of Proluton Depot have only been studied in two patients so far No changes in the plasma levels of progesterone or oestradiol were found which would indicate that the patients have received a real increase in progestogen active substances by the injection as the effect on target organs is likely to be a summation of the endogenous levels and the amount injected

It can be concluded from this study that 17α-hydroxyprogesterone caproate in a single intramuscular injection of 500 mg does not shorten the lifespan of the corpus luteum as has been found for 19-nortestosterone derivatives.

ACKNOWLEDGMENT

This study was supported by the Ford Foundation and the Population Council, New York N Y and Schering AG Berlin. The author is indebted to the devoted volunteers who made this study possible.

REFERENCES

1 Corner G. W., Jr The histological dating of the human corpus luteum of menstruation. Amer J Anat 98 377 1956.

2. Edqvist, L.-E. & Johansson, E. D. B. Radioimmunoassay of oestrone and oestradiol in human and bovine peripheral plasma. Acta Endocr (Kbh.) 1972, in press.

3 Férin, M Zimmering, P E., Liebermann, S. & Vande Wiele R. L. Inactivation of the biological effects of exogenous and endogenous estrogens by antibodies to 17β-estradiol. Endocrinology 83 565 1968

4 Fylling, P Disappearance rate of progesterone following simultaneous removal of the corpus luteum and the fetoplacental unit in women. Acta Endocr (Kbh.) 65 284 1970.

5 Hotchkiss, J Atkinson, L. E. & Knobil, E. Time course of serum estrogen and luteinizing hormone (LH) concentrations during the menstrual cycle of the rhesus monkey Endocrinology 89 177 1971

6. Johansson, E. D B. Progesterone levels in peripheral plasma during the luteal phase of the normal menstrual cycle measured by a rapid competitive protein binding technique. Acta Endocr (Kbh.) 61 592, 1969

7 — A simplified procedure for the assay of progesterone. Karolinska Symposia on Research Methods in Reproductive Endocrinology 2nd Symposium. Acta Endocr (Kbh.), Suppl. 147 188, 1970.

8. — Luteolytic effects of gestagens. Third International Congress on Hormonal Steroids, Hamburg, Germany Excerpta Medica Int. Congress Series No. 210 Abstract 459 p. 215

9 — Luteolytic effect of gestagens. Acta Obstet Gynec Scand 50 75 1971

10. — Depression of the progesterone levels in plasma in women treated with synthetic gestagens after ovulation. Acta Endocr (Kbh.) 68 779 1971

11 Johansson, E. D B & Wide, L. Periovulatory levels of plasma progesterone and luteinizing hormone in women. Acta Endocr 62 82, 1969

12. Johansson, E. D B., Wide, L & Gemzell, C. Luteinizing hormone (LH) and progesterone in plasma and LH and oestrogens in urine during 42 normal menstrual cycles. Acta Endocr (Kbh) 68 502, 1971.

13 Little B Lin, T J & Billiar R. B. The metabolic clearance rate of progesterone in the menstrual cycle and per cent interconversion to 20α-dihydroprogesterone. Excerpta Med (Amst.) Intern Congr Series No. 210 211 1970.

14 Nygren, K.-G & Johansson, E. D B Interference with the normal function of the corpus luteum by oral administration of synthetic gestagens. VIII Acta Endocr Congress, July 4–8 1971 Copenhagen. Acta Endocr (Kbh.) Suppl. 155 170, 1971 (abstract only).

15 Zander J Forbes, T R., von Münstermann, A.-M. & Neher R. Δ 3-Ketopregnene 20α-ol and Δ 3-Ketopregnene-20β-ol, two naturally occurring metabolites of progesterone. Isolation, identification, biologic activity and concentration in human tissues. J Clin Endocr 18 337 1958.

Submitted for publication June 20 1972

Elof D B. Johansson
Department of Obstetrics and Gynaecology
University Hospital
S-750 14 Uppsala 14
Sweden

Acta Obstet Gynec Scand 52: 41–46 1973

EQUIPMENT FOR DIRECT FHR MONITORING DURING LABOUR

A Comparative Artefact Study

B. Westin and G. Söderberg

From the Department of Obstetrics and Gynaecology (Head: Professor H. Johansson), Danderyds Hospital, Danderyd, and the Department of Obstetrics and Gynaecology (Head: Professor A. Ingelman-Sundberg), Sabbatsberg Hospital, Stockholm, Sweden

Abstract Electronic FHR-monitoring is, like monitoring of the heart in adults, combined with artefacts. In the first interpretation of artefacts and diagnosis of cardiac disease are facilitated, if the filtered R-wave is presented simultaneously with FHR. Changes in rate depending on artefacts can be misinterpreted with commercial instrumentations as depending on "fetal distress" which can be avoided with the equipment described in this paper. Filter properties and further reduction of noise are discussed.

Electronic FHR monitoring during delivery has not until recently been transferred from research to clinical practice. Important contributions in this field have been made by Hon (3 and 6) Caldeyro-Barcia (1) and Hammacher (2).

The recent development of relatively simple-to-operate instruments for routine obstetrical use present fetal heart rate with slow paper speed. On the one hand such slow paper speed makes it easier to interpret FHR-decelerations but on the other hand makes the interpretation of artefacts difficult. Reliable interpretation of the variations of frequency tracing cannot be performed without simultaneous recording of the trigger signal. Although efforts have been made to reduce noise in FHR-monitoring (4) discrimination in the input stage of the succeeding amplifier and filter properties may probably be improved. Attempts have therefore been made to develop an instrument that satisfies some of the criteria here presented. Comparison will be made between the equipment built by the present authors and some commercially available instruments.

TEST EQUIPMENT

Scalp electrode. At high stations and when the cervical canal was narrow the electrode used was a modified clip probe as described elsewhere (10 and 9). At other stations of the fetal head commercially available noise reducing bipolar silver-silverchloride scalp electrodes described by Hon (5) were used.

Fetal electrocardiographic signal (FECG)-amplifier and band-pass filter. Because of the high discrimination in the input stage of the succeeding amplifier we have been able to eliminate a suppressing electrode which is not customary with commercially available equipment. The FECG-signal obtained from the fetus is connected to the input of an amplifier with high discrimination and is amplified to the output level of 1 V in order to be filtered later in a narrow band-pass filter with a mean frequency of 28 Hz and steep flanks (about 30 and 12 dB per octave, respectively) (Figs. 1 and 2).

To get necessary signal level in order to drive the oscilloscope and the recorder the signal that emanates from the FECG-amplifier has been charged the input of an amplifier of standard model (EMT 12 B Elema-Schönander AB).

Heart ratemeter

This custom built instrument (EMT 30, Elema Schönander AB) consists of two units, an amplification unit and a

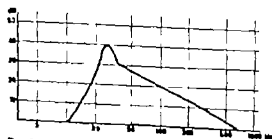


Fig. 1. Band-pass filter. Centre frequency 28 Hz.

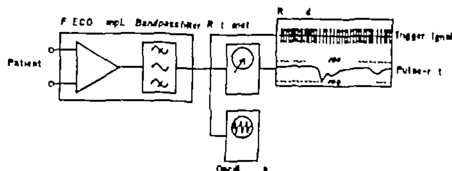


Fig Equipment for FHR-monitoring. Band-pass filter as in Fig. 1

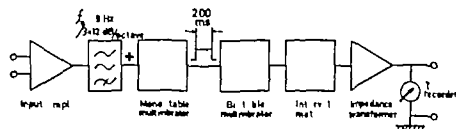


Fig. 3 Heart ratemeter. For further explanation, see text.

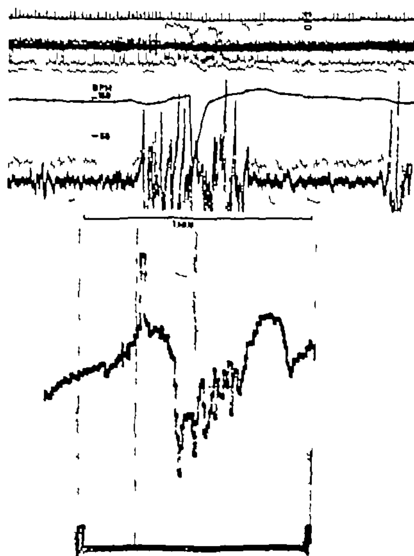


Fig. 4 Simultaneous recording from the same scalp electrode with authors equipment and Hewlett Packard (HP). Because of a too small amplitude of th trigger signal a artefact lasting f 5 sec occurred in the authors equipment (Channels and 3). Simultaneously the sign l-to-nois ratio in the HP-equipment became unfavourable for 5 sec and the tracing ga the appearance f a deceleration (Channels 4 and). Because the trigger signal is not presented the HP-equipment i not possible to interpret this deceleration as rtefact.

measuring unit. The amplifier has symmetrical input (input impedance 1 M Ohm) and an asymmetrical output. It is equipped with RC-filter elements that gives high pass characteristics with lower limit frequency of 9 Hz. This emphasizes the R-wave by amplification of the FECG-signal.

The measuring unit can be divided into two principal units: pulse unit and test unit (*Fig. 3*). The ratemeter is also equipped with loud speaker for acoustic reproduction of the FHR.

Recording units

The OSCILLOSCOPE (EMT 60 B, Elema-Schönander AB) is one-channelled oscilloscope equipped with an anti-reflex-treated screen. Sweep rates can be chosen to 10, 25 or 50 mm/sec. A suitable sweep rate has been shown to be 25 mm/sec when the oscilloscope is used for

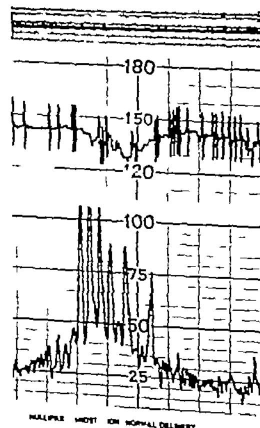


Fig. Simultaneous recording from the same scalp electrode with authors' equipment (Channels 1 and 2) and cardio-tachometer from Corometrics Med. Syst. Inc (Channels 3 FHR and 4 external tachography). Despite an entirely noise-free trigger signal in the authors' equipment (Channel 2) the tacho-cardiometer at the same time showing no less than about 30 artefacts (the vertical strokes in Channel 3).

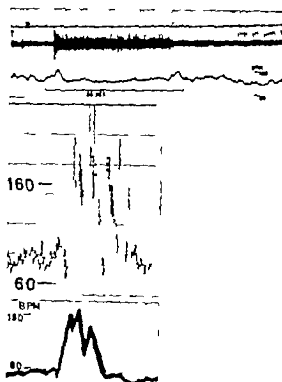


Fig. 6. Simultaneous recording with the authors' equipment and an equipment from Corometrics. When the large noise-to-signal ratio and the decrease of the amplitude of the R-wave appeared in the authors' equipment (Channel 1) the subject who was connected to the two poles of the scalp electrode took deep breath and kept her breath. The lower amplitude of the R-wave of the authors' equipment did not bring any artefacts in the heart rate (Channel 2). The heart rate (Channel 3) of the recorder of Corometrics shows only artefacts while its electronic memory (Channel 4) because of the sampling indicates an erroneous real increase of rate.

control of the signal-to-noise ratio and by pre-setting the instrument.

As result of the hard filtering the FECG-signal is greatly distorted and can in no way be compared with diagnostic FECG.

On the recorder (Mingograph 42 B or Mingograph 800, Elema-Schönander AB) the filtered FECG-signal is recorded as well as the integrated signal from the heart ratemeter in the form of an increasing respectively decreasing zero line by increasing decreasing pulse rate. Built-in electronic calibration of pulse rate is available at two different levels.

COMPARATIVE ARTEFACT STUDY

A complete description of the clinical material will be published separately.

With few cases we want to demonstrate that

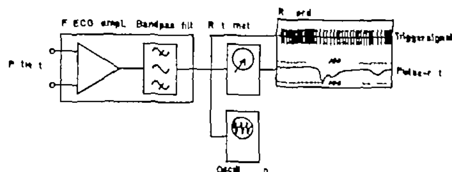


Fig Equipment for FHR-monitoring. Band-pass filter as in Fig. 1

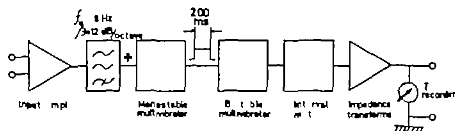


Fig 3 Heart ratemeter For further explanation, see text.

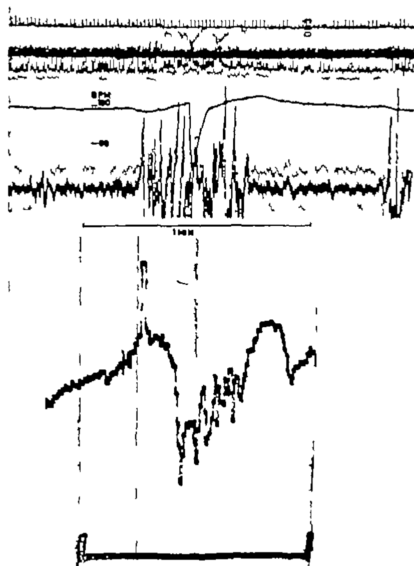


Fig 4 Simultaneous recording from the same scalp electrode with authors equipment and Hewlett Packard (HP) Because of a too small amplitude of the trigger signal an artefact lasting for 5 sec occurred in the authors equipment (Channels 2 and 3) Simultaneously the signal-to-noise ratio in the HP-equipment became unfavourable for 25 sec and the tracing ga the ppearance f deceleration (Channels 4 and 5). Because the t gge signal is not presented in the HP-equipment it is not possible to interpret this deceleration as an rtef ct.

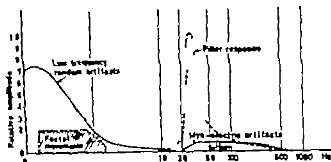


Fig 9 The spectrum of noise and artefacts (11) in fetal heart rate monitoring together with the filter response of authors equipment.

breath The lowered amplitude of the R-wave of the test equipment did not bring any artefact in the heart rate (Channel 2). The heart rate (Channel 3) of the recorder of Corometrics shows only artefacts on the recorder while its electronic memory (Channel 4) because of the sampling, indicates a real increase of rate. These results have been obtained every time in repeated experiments with several test subjects.—This model experiment reflects two common features in clinical practice, noise because of poor contact with the scalp of the fetus and low amplitude of the R-wave.

In Sweden there has been built an instrument for FHR monitoring (8) based upon commercially available standard units (Universal-amplifier AB, Elema-Schönander). The Universal-amplifier is equipped with variable filters with relatively flat flanks (about 3 dB per octave) and therefore not adapted for FECG-monitoring. In spite of this the Universal-amplifier shows (Fig 7) a trigger signal of surprisingly good quality (compare with Hewlett-Packard, Fig. 4 channel 4). Yet, with muscle activity an unfavourable signal-to-noise ratio easily appears by using the Universal-amplifier resulting in artefacts. This is not the case when using our test instrument (Fig. 7 channels 2, 3 and 5 6 respectively)

Fig. 8 (authors instrument) shows an irregular heart rate (Channel 3) which was clinically interpreted as intra-uterine asphyxia and could very well be interpreted as electronic artefacts without a simultaneous recording of filtered R-wave. With the support of R-wave-recording an irregular heart rate probably depending on supraventricular extra-systoles could be demonstrated and the delivery could continue spontaneously without complications for the fetus.

DISCUSSION

In order to reduce artefacts we have tested several similarly-shaped narrow band-pass filters with different mean frequencies. By changing the centre frequency (18 24 28 40, 55 and 78 Hz) we found empirically that a filter with a mean frequency of 28 Hz gave a better attenuation of undesired signals than filters with higher centre frequencies. Band-pass filters with lower centre frequencies than 28 Hz tended to attenuate undesired signals no better than the 28 Hz filter

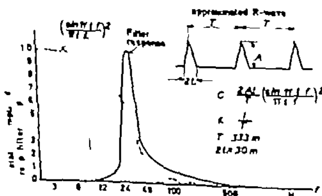


Fig 10 Fourier analyses of an approximate R-wave with duration of 30 msec and heart rate of 180 beats min together with the filter response of authors equipment.

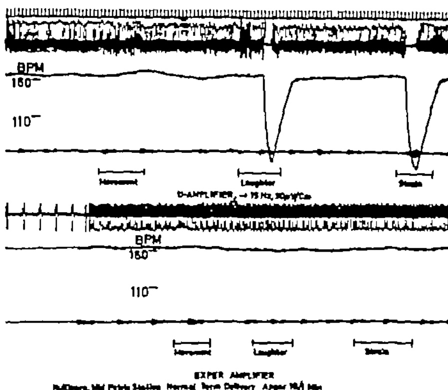


Fig 7 FHR monitoring. Comparison between standard amplifier (EMT 12B) not designed for FHR monitoring and authors equipment. EMT 12B does not attenuate myoelectrical potential from striated muscles (*upper set of records*) while this is the case with authors' equipment (lower set of records).

available commercial equipment hardly satisfies the criteria we required.

Fig. 4 shows a simultaneous recording from the same scalp electrode with the test instrument (1 = *time marking in seconds*, 2 = *filtered* R wave, 3 = FHR) and Hewlett Packard Ltd. (4 = filtered R wave 5 = FHR)

With the instrument designed by us the artefact lasted for 5 sec. The reason too small amplitude of the FECG-signal could easily be detected. The Signal to-noise ratio from the Hewlett Packard equipment, monitored simultaneously became so unfavourable for 25 seconds that the tracing gave the appearance of a deceleration. It is beyond doubt that this is an artefact but the reason why *there is a relatively long deceleration* on the Hewlett Packard monitoring equipment can not be determined as the filtered FECG-signal is not recorded on the paper of this equipment.

Fig. 5 is a simultaneous recording from the same scalp electrode with the test equipment (Channels 1 = seconds and 2 = trigger signal) and a cardio-tachometer from Corometrics Med. Systems Inc. (3 = *fetal heart rate* 4 = *external tocography*) Despite of an entirely noise-free trigger signal in the test equipment the cardio-tachometer is at the same time showing no less than about 30 artefacts (the vertical strokes) This suggests among other things sensitivity to the amplitude of the trigger signal. Fig. 6 shows that this is the case. The test equipment (1 = filtered R wave 2 = FHR) Corometrics Med. Systems Inc (3 = *FHR* 4 = *FHR on central supervision unit, 8 min* electronic memory) When the large noise-to-signal ratio and the decrease of the amplitude of the R-wave appeared (Channel 1) a test subject who was connected to the two poles of the scalp electrode took a deep breath and kept her

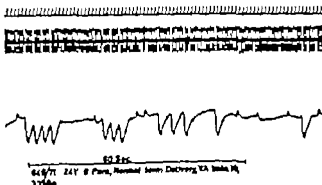


Fig 8 Irregular heart rate clinically interpreted as intrauterine asphyxia. By FHR monitoring an irregular rate could very well be interpreted as electronic artefacts without a simultaneous recording of filtered R-wave. Heart rate calibration. 70 beats/10 mm.

Acta Obstet Gynec Scand 52: 47–50 1973

FETAL AND MATERNAL pH MEASUREMENTS

A Basis for Common Normal Values

G Rooth, R. McBride and B J Ivy

From the Department of Obstetrics and Gynecology (Head. R. P Beck), University J Alberta, Edmonton, Alberta, Canada

Abstract Maternal and fetal acid-base changes in series of 36 normal cases from Edmonton, Canada, are compared with similar series from Lund, Sweden, published by Jacobson. During labour, pH in the mother and in the fetus falls in Lund, but not in Edmonton. The mothers labour hard in Lund and are more sedated in Edmonton. The pH difference (ΔpH) between the maternal and fetal blood is about 0.10 in both series.

In another series of 41 cases classified as abnormal because of changes in fetal heart rate or meconium staining of the amniotic fluid, the mean ΔpH was 0.15. ΔpH was correlated to the fetal scalp pO_2 and oxygen saturation and there was significant drop in fetal oxygenation when ΔpH increased.

It is suggested that fetal preacidosis be redefined as ΔpH 0.15–0.19 and acidosis ΔpH > 0.20 pH units.

Like any living organism, the fetus must eliminate its surplus of hydrogen ions. This is done via the umbilical circulation to the mother. The maternal pH will not be appreciably influenced by the number of hydrogen ions coming from the fetus, but the fetal pH will be influenced by the maternal level as the fetal hydrogen ions need a sufficient electro-chemical gradient to be eliminated. It has been shown that during normal delivery the fetal pH fluctuates parallel with that of the mother whether the maternal pH is increased by administration of sodium bicarbonate, or decreased by ammonium chloride or tissue hypoxia (2, 3 5 9). The difference between maternal and fetal pH is about 0.10 pH units in several series: this then appears to be the difference needed for adequate fetal hydrogen ion elimination under normal circumstances. Increased values would then indicate chemical signs of fetal asphyxia.

The discrepancy between fetal pH levels in different centres (8) may to a large extent be accounted for by different maternal pH levels due to variations in the clinical material or in the management of the patients. Comparisons between different series should include the whole course of labour as pH may vary significantly during labour. The present series of maternal and fetal acid-base data is compared with a Swedish series in order to try to find an approach to normal values which could be valid in different centres.

The interrelation between maternal and fetal acid-base balance may be explained on the assumption that a mean pH difference of about 0.10 pH units has to be maintained (6) and this value increases with increasingly severe signs of abnormal fetal heart rate patterns (7). As the oxygen supply to the fetus should be in jeopardy when the hydrogen elimination is impeded, we have also studied the correlation between the maternal-fetal pH gradient and the fetal oxygen tension.

MATERIAL

As one objective to the series was the gaining of local experience by the fetal scalp sampling technique, relatively many clinically "normal" patients were studied. 64 fetal scalp samples were obtained from 36 fetuses classified as "normal" i.e. delivery was at term, the mother had no diseases increasing the risk of the outcome and labour was uncomplicated and the one minute Apgar score > 7. In 50 cases, maternal venous blood was taken from cubital vein at the same time as the fetal scalp blood.

64 fetal scalp blood samples were taken from 61 fetuses either showing abnormal fetal heart rates (FHR) or meconium staining of the amniotic fluid or both these signs. Of these patients, 15 had an Apgar score < 7 (mean 5.0).

The spectrum of noise and artefacts in fetal heart rate monitoring together with the filter response of our equipment (centre frequency 28 Hz) is illustrated in Fig. 9 Moving the mean fre quency of the filter towards lower frequencies implies some risk of low frequency artefacts. It is also evident that moving the mean frequency of the filter towards higher frequencies involves an increased risk of myo-electric artefacts.

A Fourier analyses of an approximate R wave with a duration of 30 m sec and a heart rate of 180 beats/min is illustrated in Fig. 10 together with the filter response. It is seen that sufficient amounts of energy of the approximated R wave passes the filter while undesired signals are at tenuated The present filter might be modified by broadening the pass band towards lower fre quencies and by further reduction of hum and myo-electrical artefacts.

Improvement of beat to-beat heart rate meters may be obtained by more sophisticated trigger circuits. Lindborg et al. (7) have designed a beat to-beat heart rate meter with linear analogue output and special designs to avoid false triggering due to disturbance potentials from striated muscles. It is also insensitive to variation in the ECG signal level which further reduces the risk of artefacts.

ACKNOWLEDGEMENTS

This work was supported by a grant no. B71 17X 773 03B from the Swedish Medical Research Council. We are indebted to Mr Claes Lundgren for valuable technical assistance and to Mr Torsten Aström, M.S. for fruitf l discussions on filter properties.

REFERENCES

1 Caldeyro-Barcia, R., Mendez Bauer C., Poseiro, J J Escarcena, L. A., Pose S. V Bieniarz, J Arnt, L. Gulin, L. & Althabe O. Control of human fetal heart rate during labor In Th. Heart and Circulation in the Newborn and Infant, pp. 7 36. Grune & Stratton, New York, 1966.

2. Hammacher K.. In Perinatal Medicine. 1st European Congress, Berlin, 1969 George Thieme Verlag, Stuttgart.

3. Hon, E. H.. The classification of fetal heart rate I. A working classification. Obstet Gynec 137 1963.

4 Hon, E. H. & Lee S. T.. Noise reduction in fetal electrocardiography Am J Obstet Gynec 87 1086, 1963

5 Hon, E. H.. A fetal electrocardiographic electrode. Yale J Biol Med 39 54 1966.

6. Hon, E. H.. An Atlas of Fetal Heart Patterns. New Haven, Conn., Harty Press Inc., 1968.

7 Lindborg, B., Wigertz, O. & Ödman, Th.. A beat-to-beat heart rate meter with linear analog output for muscula exercise studies. Research Groups on Aviation and Naval Medicine of the Swedish Med. Research Council, Karolinska Institutet, Stockholm, 1969

8. Peterson, H., Tyreman, N O & Anberg, A.. Kardiotakograf för fosterövervakning under förlossning. S Läkartidn 68 4387 1971

9 Unger F W & Goodwin, J W.. Instrumentation for fetal electrocardiography and intrauterine pressure A new scalp electrode and radiotelemetry system. Am J Obstet Gynec 11 351 1972.

10. Westin, B Söderberg, G & Lundgren, C.. Apparatur för övervakning v fosterhjärt-frek ens under förlossning. Swedish Med. Research Council, Karolinska Institutet, Stockholm, 1971

11 White D A. & Trenholm, B. G Reliable triggering for exercise electrocardiograms. IEEE Trans Biomed Engin 16. 75 1969

Submitted for publication June 30, 197

Björn Westin
Department of Obstetrics and Gynaecology
Danderyd Hospital
Danderyd
Sweden

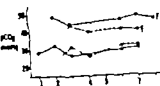


Fig. 5. Maternal and fetal pCO_2 in Edmonton in normal cases (O- -O) and in cases with abnormal fetal heart rate or meconium-stained amniotic fluid (●—●)

The maternal-fetal pH difference or ΔpH was greater in the risk group than in the normal cases, as seen from Fig. 4 and plotting ΔpH against the fetal oxygen tension from the 31 cases in which simultaneous measurements were done, gives the negative statistically highly significant correlation in Fig. 7. If the pO_2 values are re-calculated to oxygen saturation using the in vivo fetal oxygen dissociation curve (13) the following relation was found: oxygen saturation = 81 − 273 ΔpH, $r = -0.72$, $p < 0.001$.

It follows that at a ΔpH of 0.10 pH units the fetal scalp oxygen saturation is about 55% but falls to 40% when ΔpH increases to 0.15. The mean ΔpH in the 15 cases with an Apgar score 7 was 0.15 ± 0.08. As pH measured during the first stage of labour does not have to be correlated with the Apgar score taken after birth a better correlation would be expected between ΔpH in the second stage of labour and Apgar score. In the 8 cases observed ΔpH was then 0.19 ± 0.06.

DISCUSSION

The comparison between the results in Edmonton, Canada and Lund, Sweden illustrates the effect of different obstetrical procedures and emphasizes that "normal" values should be obtained locally.

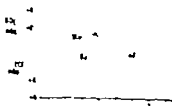


Fig. Maternal and fetal Base deficit in Edmonton in normal cases (O- -O) and in cases with abnormal FHR or meconium-stained amniotic fluid (●)

4 · 731941

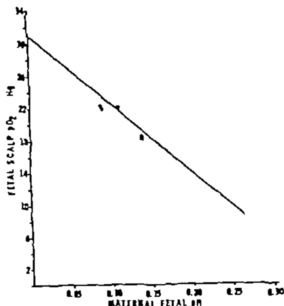


Fig. 7. Correlation between ΔpH (maternal-fetal pH) and fetal scalp pO_2 in Edmonton, $pO_2 = 31.2 - 85$ ΔpH, $r = -0.$ 31, p 0.001

The lack of agreement between normal values for fetal scalp blood can in the present series be explained by the metabolic acidosis of the mothers during labour in Lund and virtual absence of this in Edmonton, where all the mothers were given Demerol and additional anesthesia. In Lund, the women labour hard and local tissue hypoxia is the result. In Edmonton, this was not the case.

In Lund, it was observed that the pH and oxygen saturation in the cord blood was lower when the mothers had obtained Demerol than in normal cases (10). Demerol was there only given for prolonged, difficult labour when the mothers needed rest or for similar complications. Consequently in Lund Demerol was found to be a negative factor as opposed to Edmonton.

Saling (14) and several subsequent workers called values between 7.25 and 7.20 pre-acidosis, and < 7.20 acidosis. According to this definition, the mean pH in the Edmonton risk group is "normal" throughout labour and only in seven cases were values seen between 7.25 and 7.20 and in only one case was there a value of 7.12. Using such criteria, the fetal scalp blood would only have implicated fetal risk in the form of acidosis

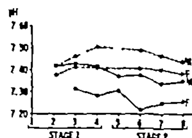


Fig 1 Maternal and fetal pH in Lund (●—●) and Edmonton (O- -O) during delivery

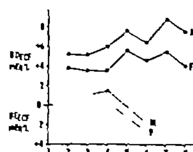


Fig 3 Maternal and fetal Base deficit in Lund (●—●) and Edmonton (O- -O) during delivery

The mothers were delivered according to the practice of the hospital. They were given Demerol in doses varying from 50 to 1.5 mg plus Phenergan 25 to 50 mg and in addition (?) paracervical, (10) epidural anesthesia, (32) pudendal block, and (18) short "whiffs" of 50% N_2O and O_2.

The Edmonton material is compared with that of Jacobson (4) from Lund, Sweden, using his N series which is "normal" by the same definitions as used here. In both these series the maternal blood sample was from a cubital vein. The progress of delivery is classified 1–8 according to Jacobson. Stage 1 of labour is 1–4 corresponding to 2 10 centimeters of dilatation. 5 is fully dilated and onset of second stage of labour 6 is during second stage of labour 7 is 15–5 minutes before delivery and 8 is within 5 minutes of delivery

METHODS

pH, pCO_2 and pO_2 were measured with an Instrumentation Laboratories blood-gas unit. The fetal scalp samples were taken with the Saling technique (14) in capillaries, and analyses were usually performed within 10, always within 15 minutes of sampling. Blood was drawn between contractions, as a rule. Although pH was measured in all samples, there was often insufficient blood to analyse for pCO_2 and for pO_2 at the same time. The pH reference standard is based on U.S. pH standards. Base excess or Base deficit of the extracellular fluid (BD_{ecf}) was calculated from the Base excess nomogram of Siggaard-Andersen (15). BD_{ecf} has the advantage of not being influenced by in vivo increases in pCO (11).

RESULTS

Fig. 1 shows that both the maternal and fetal pH is higher in Edmonton than in Lund. It will be seen that the mean normal fetal pH in Edmonton is close to 7 40 throughout labour and the significant drop in pH seen during the second stage in Lund is absent in Edmonton.

Fig. 2 demonstrates that the maternal pCO values are similar in Edmonton and Lund whereas fetal pCO is higher in Lund than in Edmonton.

Fig. 3 illustrates that the main reason for the pH differences between Edmonton and Lund lies in the observation that the Base deficit present in Lund is minimal or absent in Edmonton.

Comparing (in Fig 4) the normal Edmonton material with the group showing signs of intrauterine distress, it will be seen that the mean maternal pH values do not differ whereas the mean pH value of the fetuses at risk is 0 05 pH units lower than in the normal group. This is partly explained by higher pCO_2 in the risk group (Fig. 5) Insufficient data were available to obtain a curve for Base deficit in the risk group, but Fig. 6 shows that in the risk group the fetus has more Base deficit than the mother which is just the opposite of what was seen in the normal group. The mean fetal scalp pO_2 in the normal case was about 4 mmHg higher than in the risk group and tended to fall slowly during labour whereas it remained stable at about 20 mmHg in the risk group.

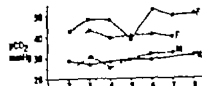


Fig 2 Maternal and fetal pCO_2 in Lund (●—●) and Edmonton (O- -O) during delivery

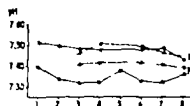


Fig 4 Maternal and fetal pH Edmonton in normal cases (O- O) and in cases with abnormal FHR or meconium-stained amniotic fluid (●—●).

Acta Obstet Gynec Scand 52: 51–57 1973

PURIFICATION OF THE "PREGNANCY ZONE" PROTEIN

Bo von Schoultz and Torgny Stigbrand

From the Departments of Obstetrics and Gynecology (Head. Prof. Per Lundström) and Medical Chemistry (Head. Prof. K.-G. Paul), University of Umeå, Sweden

Abstract. The "pregnancy zone" protein is a serum protein found in pregnancy and after administration of oral contraceptives. It has hitherto not been isolated and therefore practically nothing is known about its structure and function. Purification of this protein was achieved in three steps: 1) Gel filtration on Sephadex G-200; 2) Chromatography on DEAE-Sephadex; 3) Preparative polyacrylamide electrophoresis. After the third step a protein was obtained which was found to be pure judged by the behaviour in polyacrylamide electrophoresis and ultracentrifugation. The molecular weight was estimated to be 324 000, the sedimentation coefficient 12 S and the isoelectric point 4.7.

By means of vertical starch gel electrophoresis Smithies (10) found a protein zone in about 10% of serum samples from pregnant women. This zone which migrated among the haptoglobin bands between the slow α macroglobulin and transferrin was not found in non-pregnant women or in men. Afonso and Farnham (2) reported that this "pregnancy zone" protein, which was lacking in cord serum, was found in 82% of women at term of pregnancy and that it disappeared from serum during the puerperium. Furthermore the "pregnancy zone" was found to appear with increasing frequency during pregnancy between 9 weeks gestation when it was first seen and 25 weeks when it was present in about 80% of the serum samples examined (1).

The detection of the "pregnancy zone" is dependent on the haptoglobin type: the detectability decreases in the order Hp 1-1, Hp 2-2, Hp 2-1 (4). The "pregnancy zone" has been found in a higher frequency among women carrying a female fetus. The reason for this was not clear (4). Presence of "pregnancy zone" did not seem to affect fetal welfare (1, 4). It was not visible in cases of gynecological malignancy (1). The protein was found to be distinct from transcortin, thyroxin-binding globulin and ceruloplasmin (1).

The oral contraceptive drug Enovid (10 mg Norethynodrel, 0.15 mg Mestranol) was found to induce the "pregnancy zone" in 92% of treated women. It disappeared after stopping the drug (3).

In later studies a significant difference was found with respect to the frequency with which the "pregnancy zone" was induced by different types of oral contraceptives (6).

The "pregnancy zone" protein has hitherto not been characterized chemically and therefore practically nothing is known about its structure and function. This work describes the isolation of the "pregnancy zone" protein which is a necessary prerequisite for the further elucidation of its physiological role.

MATERIAL AND METHODS

Serum samples from pregnant women were tested for haptoglobin type and presence of the "pregnancy zone" by means of discontinuous polyacrylamide gel electrophoresis (7.5% polyacrylamide) according to Davis (8). Hemoglobin sufficient to saturate the haptoglobins was added to the sera and the electrophoresis was run for four hours in gel tubes 5.5 × 90 mm with 100 V and 3 mA per tube. The gels were stained for haptoglobin bands with benzidine and H_2O_2 and with amido-black. Destaining was made electrophoretically before inspection.

Retroplacental blood of haptoglobin type 1-1 was taken at delivery from women who during late pregnancy had demonstrated a heavy "pregnancy zone". The serum was stored at −15°C until use.

Column chromatography was performed with Sephadex G-200 and DEAE Sephadex purchased from AB Pharmacia (Uppsala and Umeå, Sweden) and conditioned as prescribed by the manufacturer. All work was performed at +4°C.

Preparative polyacrylamide gel electrophoresis, Uniphor LKB, Stockholm, Sweden, was used according to the manufacturers' instructions.

Ultracentrifugations were made at 20°C in a Beckman Model E analytical ultracentrifuge equipped with an

or pre-acidosis in 11% in this Canadian material The reason for this low percentage is obviously the initially high pH in the mothers and the fetuses in Edmonton More important than an absolute level is a drop in pH and a fall amounting to 0 10 or 0 15 pH units would still leave the pH above 7 25 This drop would b. considered a sign of fetal distress by most people using the fetal scalp technique

The high fetal pH found in the present series is due to the high maternal pH but it will be observed that the normal maternal-fetal pH is about 0 10 pH units, and this is the same figure as published in other series. As the maternal pH level may occasionally be affected by a temporary hyper or hypoventilation ΔpH must in a certain percentage be misleading too It is possible that more accurate information is obtained by studying the difference between the maternal and fetal Base deficit (1) or even better of course would be a study of both the maternal and fetal complete acid-base changes by repeated sampling (12) That however calls for additional measurements of pCO_2 and calculation of Base deficit and an extensive experience with acid-base para meters, which as yet not all obstetricians have

The clinical value of ΔpH is seen from the al ready mentioned fact that there is a good cor relation between the increase in ΔpH and the severity of abnormal heart rate patterns, and now we have found that the fetal oxygen supply de creases when ΔpH increases.

If fetal *pre-acidosis* is redefined as a fetal pH being 0 15 to 0 19 pH units below that of th maternal pH, and *fetal acidosis* the situation when the fetal pH is 0 20 or more pH units below the maternal level then it would seem that pH measurements give more information and we can hope that the same standards could be used in different centres.

ACKNOWLEDGEMENT

Supported from a grant fr m Medical Research Council of Canada.

REFERENCES

1 Beard, R. W Morris, E. D & Clayton, S. G Fetal blood sampling in clinical obstetrics, J Obstet Gynaec Brit Comm *73* 56., 1966

2 Blechner J N Stenger V G., Eitzman, D. V & Prystowsky H. Oxygenation of the human fetus and newborn infant during maternal metabolic acidosis. Amer J Obstet Gynec 108 47 1970.

3 Goodlin, R. C. & Kaiser I. H. The effect of ammonium chloride induced maternal acidosis on the human fetus at term. pH, hemoglobin, blood gases. Amer J Med Sci .33 66., 1957

4 Jacobson, L. Studies on acid-base and electrolyte components of human foetal and maternal blood during labour Studentlitteratur Lund, 1970, pp. 1 156.

5 Jacobson, L. & Rooth, G. Sodium bicarbonate administration during labour Th. effect on the electrolytes and acid-base balance of mother and foetus. *In* Intra-Uterine Dangers to th. Foetus, p. 417 Excerpta Medica Foundation, Amsterdam, 1968.

6. J cobson, L. & Rooth, G. Interpretati e aspects on the acid-base composition and it ariation in fetal scalp blood and matern l blood during labour J Obstet Gynaec Brit Comm *78* 971 1971

7 Khann, A. F & Hon, E. H. Observations on fetal heart rate and fetal biochemistry II Fetal-maternal pH differences. Amer J Obstet Gynec *109* 41., 1971

8 Lumley J & McKinnon, L. Lack of agreement on normal values for fetal scalp blood. J Obstet Gynaec Brit Comm *78* 13 1971

9 Rooth, G Early detection and prevention of foetal acidosis. Lancet *1* 290 1964

10 Rooth, G Unpublished results.

11 Rooth G & Jacobson, L. The value and ability of *Base Excess* in perinatal acid-base studies. Scand J Clin Lab Invest *3* 283 1971

12 Rooth, G Jacobson, L., Heinrich, J & Seidenschnur G The acid-base status of th. fetus during normal labou Presented t th. Symposium on Placental Gas Exchange Hannover 1971 (ed L Longo). Washington, 1972. I press.

13 Rooth, G., Sjöstedt, S. & Caligara, F The m i foetal oxygen dissociation curve Biol Neonat *1* 61 1959

14 Saling, E. Das Kind im Bereich de Geburtshilfe. Georg Thieme Verlag, Stuttgart, 1966.

15 Siggaard-Andersen, O The pH log pCO blood acid-base nomogram revised Scand J Clin Lab Invest *14* 598, 196.

S bmitted for publication Jul 5 1972

Gosta Rooth
Research Department I
E-blocket
Uni ersity Hospital
Lund
S eden

cm Sephadex G-200 column and eluted with 30 mM sodium phosphate pH 7.4. Protein was determined spectrophotometrically by light absorption at 280 nM (Fig. 3). The "pregnancy zone" protein was identified in the right part of the first of the three main peaks, by polyacrylamide gel electrophoresis.

Step 2. Fractions in the shaded area in Fig. 3 were pooled (approx. 25 ml) and applied on a 1.5 × 12 cm DEAE-Sephadex ionic exchanger equilibrated with 30 mM sodium-phosphate pH 7.4. A slight white precipitate was seen on top of the column. A linear NaCl-gradient (0.0–0.3 M) in the same buffer was applied. The "pregnancy zone" protein was obtained in the main sharp peak *after* the gradient was started (Fig. 4).

Step 3. Fractions within the shaded area in Fig. 4 were pooled (approx. 15 ml) and dialyzed overnight against glass distilled water and then concentrated to approx. 3 ml by collodium-ultrafiltration. The sample was then applied on top of the preparative gel (6.3% polyacrylamide gel 5 ⌀ × 5 cm in a discontinuous buffer system, top buffer Tris-glycin pH 8.3 and bottom buffer Tris-HCl pH 8.1). The bottom buffer was used as elution fluid. Before electrophoresis the column was run without sample for 2 hours at 200 V 10 mA. After application the sample was allowed to enter the gel for 2 hours at 200 V 10 mA and then electrophoresis was performed for 36 hours at 600 V 20 mA. Temperature in the water jacketed cooling mantle +20 C. Elution flowrate was 6 ml/hour and 3 ml fractions were collected. The "pregnancy zone" protein left the gel as a separate peak after approx. 30 hours, as shown by the arrow in Fig. 5.



Fig. 2. Polyacrylamide gel electrophoresis of serum samples from a woman during the third trimester of pregnancy (B) and three months after delivery (A). *Alb* albumin, *Tf* transferrin; *PZ*, "pregnancy zone" protein. The arrow shows the direction of migration towards the anode.

Purity and yield

For each step in the purification procedure analytical polyacrylamide gel electrophoresis was performed (Fig. 6). After step 3 only one band (the "pregnancy zone") was found on the gel.

The protein was judged as pure from the observation of a single band on polyacrylamide gel electrophoresis, homogenous sedimentation behaviour and a straight line obtained after equilibrium sedimentation.

Quantitative protein determination revealed that the yield was 1.2 mg "pregnancy zone" protein from 70 ml retroplacental serum.

Molecular weight and isoelectric point

The purified protein was subjected to analytical ultracentrifugation. The determinations of the molecular weight gave Mw = 326 000 at three different concentrations and linear relationships were obtained when log *c* was plotted versus r^2. Fig. 7 shows the experiments conducted at 70 000 rpm. From the slope of the plot the molecular weight of the protein can be computed. The partial specific volume of the protein was taken to 0.75 ml/g.

The sedimentation coefficient was 12 S measured at the same concentration as the molecular weight determinations. Only one single homo-

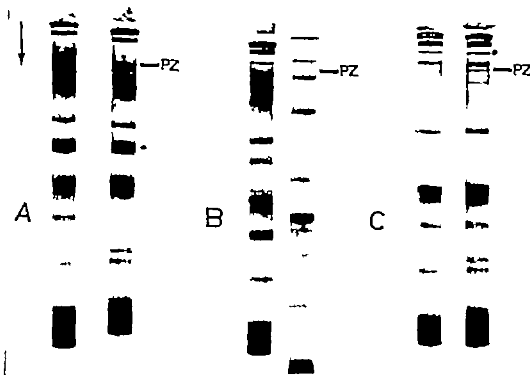


Fig 1 Polyacrylamide gel electrophoresis showing the presence of the "pregnancy zone" protein (*PZ*) in serum samples of the three main haptoglobin types. (A) Hp 1 1 (B) 2 1 (C) 2 2. The haptoglobin bands are indicated by black dots. The right gel in each pair shows the "pregnancy zone" protein. The arrow shows the direction of migration towards the anode.

RTIC temperature control unit and an electronic speed control. All samples were dissolved in 0 1 M Tris-HCl buffer pH 8.1 Six-channel epon-filled Yphantis center piece was used for the molecular weight determinations. The sedimentation coefficient at 0.5 mg/ml protein concentration was measured with the use of 12 mm double sector cells. Sapphire windows were used throughout. Recordings were made with both Schlieren and Rayleigh interference optics. The sedimentation equilibrium experiments were performed with the meniscus depletion technique of Yphantis (11). Fringe displacements at given x-coordinates were measured on photographs taken at different time intervals. Calculations of apparent average molecular weights were computed according to Schachman (9).

Isoelectric focusing was performed for 48 hours, at 600 V in the LKB column 8101 110 ml (LKB Stockholm, Sweden) with 1% Ampholine according to LKB' suggestions and with sucrose density gradient. The pH gradient covered the interval 3–6.

Protein was determined spectrophotometrically by measuring the absorption at 280 nm.

RESULTS

Demonstration of the "pregnancy zone"

The presence of the "pregnancy zone" in sera of the three main haptoglobin types is shown in Fig 1

In sera of the haptoglobin type 1 1 the "pregnancy zone" is the only protein present in higher concentration in this area of the gel and the "pregnancy zone" is therefore easily detectable. *In the haptoglobin type 2 2 the "pregnancy zone"* migrates just in front of the second haptoglobin band, but it can still be identified. In sera of the haptoglobin type 2 1 detection may be difficult, since the "pregnancy zone" migrates just behind the third haptoglobin band (counting from the anode) Serum of haptoglobin type 1 1 was preferred in the purification procedure. Fig. 2 shows *the electrophoretic patterns of serum samples from a woman with the haptoglobin type 1 1 in* late pregnancy when she had a heavy "pregnancy zone" and three months after delivery when the "pregnancy zone" had disappeared.

Purification

The purification was performed according to the following procedure

Step 1 Two times ten ml of retroplacental serum of haptoglobin type 1 1 showing the "pregnancy zone" protein was applied on a 2.8 × 110

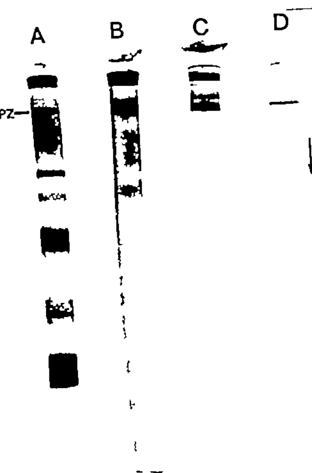


Fig. 6. Analytical polyacrylamide gel electrophoresis performed for each step of the purification procedure. From the left is shown the protein pattern in retroplacental haptoglobin 1-1 serum (A), after Sephadex G-200 chromatography (B), after DEAE Sephadex (C) and finally the isolated "pregnancy zone" protein obtained from preparative polyacrylamide gel electrophoresis (D). The arrow shows the direction of migration towards the anode.

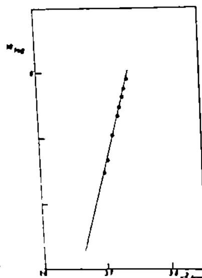
genous peak—as shown in Fig. 8—was obtained in agreement with the molecular weight determinations.

Isoelectric focusing was performed in pH region 3–6 showing one main peak eluted with a fraction at pH 4.7 (Fig. 9).

DISCUSSION

In the purification of the "pregnancy zone" protein it is important to use sera of haptoglobin type 1-1 as starting material in order to avoid

Fig. 7. Determination of molecular weight of "pregnancy zone" protein in 120 mM Tris-HCl, pH 8.1. Experiments were performed at 0.5, 0.25 and 0.15 mg/ml concentration. ... denotes the distance from the centre of rotation. The speed for these analyses was 20 000 rev. per min.



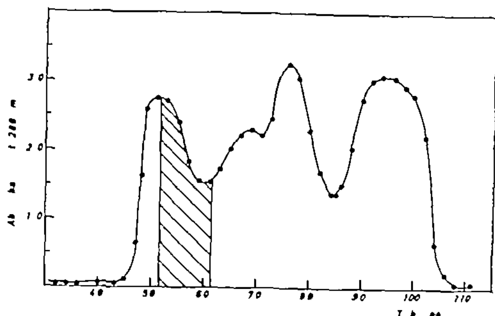


Fig 3 Gel filtration on Sephadex G-200 (2.8 110 cm, 30 mM sodium phosphate pH 7.4) of 10 ml haptoglobin 1 1 serum from a pregnant woman. Flow rate 6 ml/hour 3.0 ml fractions were collected. ●—● Absorbance at 280 nm. The "pregnancy zone protein was eluted within the shaded area.

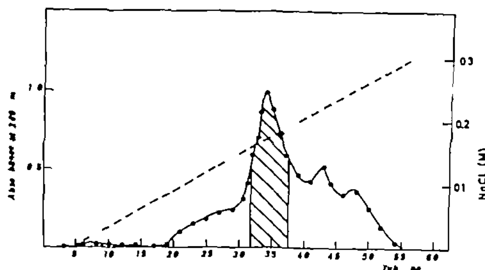


Fig 4 DEAE Sephadex chromatography (1.5 12 cm, 30 mM sodium phosphate pH 7.4) on fractions obtained *within shaded area in Fig. 3.* 3.0 ml fractions were collected. Concentration of the linear NaCl-gradient; ●—● Absorbance at 280 nm. The pregnancy zone was eluted within the shaded area.

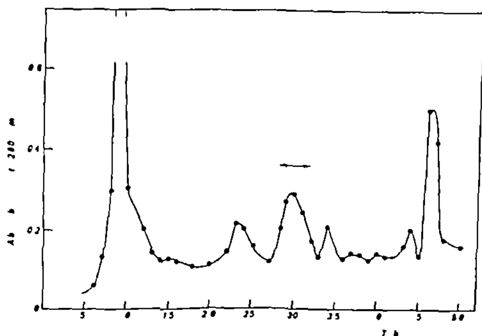


Fig 5 Discontinuous preparative polyacrylamide gel lectrophoresis with 6.30 % polyacrylamide gel (5 5 cm) on fractions obtained within shaded area n Fig. 4 after concentration and dialysis. *Flow rat 6 ml/hour* ●—● Absorbance at 280 nm. The isolated "pregnancy zone protein is shown by the arrow

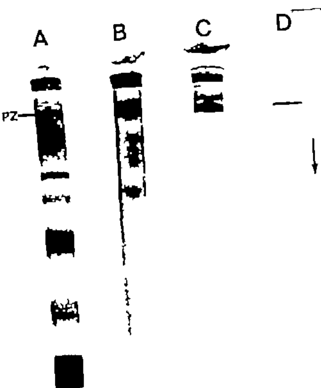


Fig. 6. Analytical polyacrylamide gel electrophoresis performed for each step of the purification procedure. From the left is shown the protein pattern in retroplacental haptoglobin 1 1 serum (A), after Sephadex G 200 chromatography (B), after DEAE Sephadex (C) and finally the isolated "pregnancy zone" protein obtained from preparative polyacrylamide gel electrophoresis (D). The arrow shows the direction of migration towards the anode.

genous peak—as shown in Fig. 8—was obtained in agreement with the molecular weight determinations.

Isoelectric focusing was performed in pH region 3–6 showing one main peak eluted with a fraction at pH 4.7 (Fig. 9).

DISCUSSION

In the purification of the "pregnancy zone" protein it is important to use sera of haptoglobin type 1 1 as starting material in order to avoid

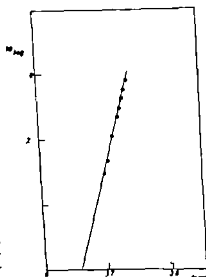


Fig. 7. Determination of molecular weight of "pregnancy zone" protein in 120 mM Tris-HCl, pH 8.1. Experiments were performed at 0.5, 0.25, and 0.15 mg/ml concentration. r denotes the distance from the centre of rotation. The speed for these analyses was 20 000 rev. per min.

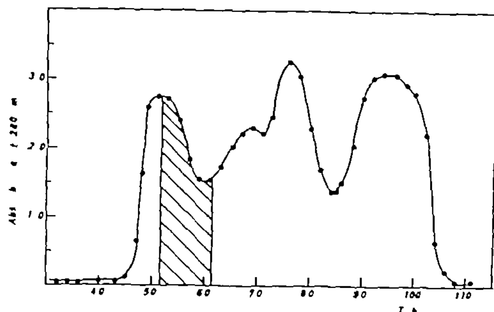


Fig. 3. Gel filtration on Sephadex G-200 (2.8 × 110 cm, 30 mM sodium phosphate pH 7.4) of 10 ml haptoglobin 1-1 serum from a pregnant woman. Flow rate 6 ml/hour. 3.0 ml fractions were collected. ●—● Absorbance at 280 nm. The "pregnancy zone protein" was eluted within the shaded area.

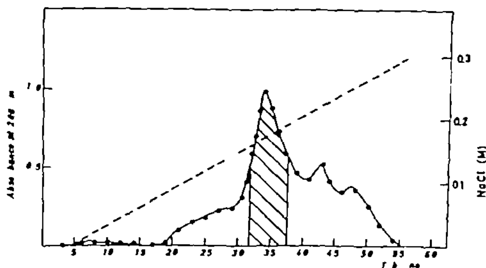


Fig. 4. DEAE-Sephadex chromatography (1.5 × 12 cm, 30 mM sodium phosphate pH 7.4) on fractions obtained within shaded area in Fig. 3. 3.0 ml fractions were collected. ---- Concentration of the linear NaCl-gradient. ●—● Absorbance at 280 nm. The pregnancy zone was eluted within the shaded area.

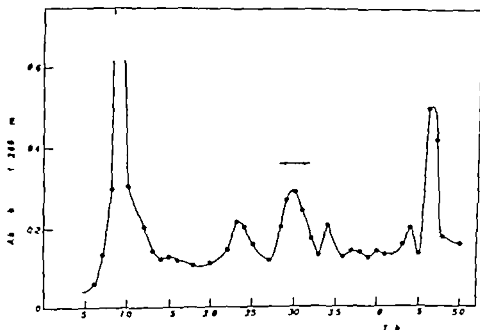


Fig. 5. Discontinuous preparative polyacrylamide gel electrophoresis with 6.30% polyacrylamide gel (5 × 5 cm) on fractions obtained within shaded area in Fig. 4 after concentration and dialysis. Flow rate 6 ml/hour. ●—● Absorbance at 280 nm. The isolated pregnancy zone protein is shown by the arrow.

The "pregnancy zone" protein is not of placental origin, since it has been found in women treated with oral contraceptive drugs. The organ of origin is unknown, but with the development of an immunological reagent to the "pregnancy zone" protein it should be possible to solve this problem.

ACKNOWLEDGEMENTS

We wish to express our gratitude to Professor L. Beckman and P Lundström and Dr N. G. Holmberg for stimulating criticism and advice and to Mrs Kerstin Hjortsberg and Mrs Kat White for skilled technical assistance. This work was supported by grants from the Medical Faculty University of Umeå.

REFERENCES

1. Afonso, J F & de Alvarez, R. R. Am J Obstet Gyn *89* 204 1964.
2. Afonso, J F & Farnham, N. G. Am J Obstet Gyn *84* 199 1962.
3. de Alvarez, R. R. & Afonso, J F Penn Med J *70* 43 1967
4. Beckman, L., Beckman, G. & Stigbrand, T Human Hered *20* 530, 1970.
5. *Beckman, L., Björling, G. & Christodoulou: Acta* Genet *16*: 122, 1966.
6. Beckman, L., von Schoultz, B and Stigbrand, T Acta Obstet Gynec Scand *50*, 369 1971
7. Boyer, S. H.: Science *134* 1002, 1961.
8. Davis, B. J N. Y Acad Science *121* 404, 1964.
9. Schachman, H. K. Ultracentrifugation in Biochemistry Academic Press, New York, 1959
10. Smithies, O.: Advances Protein Chem *14* 65 1959
11. Yphantis, D. A. Biochem *3* 297 1964

Submitted for publication July 26, 1972

Bo von Schoultz
Department of Obstetrics and Gynaecology
University
901 85 Umeå
Sweden

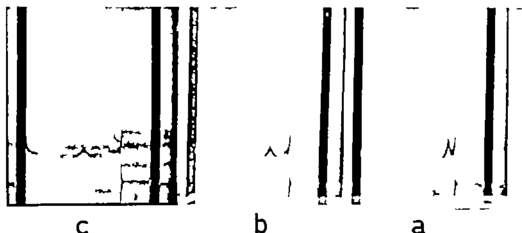


Fig 8 Schlieren pattern from sedimentation velocity experiments on the purified pregnancy zone protein conducted at 56 000 r.p.m. The protein concentration 0.5 mg/ml in 0.120 M Tris-HCl pH 8 1 Pictures were taken at (*a*) 4 min, (*b*) 12 min, (*c*) 24 min.

contamination by the polymerizing type of haptoglobin. Throughout the isolation procedure mild techniques were used. The pH varied between 7 4–8 1 and the salt concentration did not exceed 0.3 M NaCl. No precipitation techniques with organic solvents were used. Thus in all probability we have been able to purify the native "pregnancy zone" protein, which is a high molecular weight protein (326 000) with an isoelectric point of 4 7

The recovery with this method could be roughly estimated to be 20–30% and the preparation can be made within 10 days. Further studies on the chemical characterization are under way as well as immunization experiments in rabbits by means of purified protein. The purification of the "pregnancy zone" protein should make it possible to elucidate the functional role of the pregnancy zone protein

Several normally occurring plasma proteins show changes in their concentrations during pregnancy Pregnancy proteins, however represent a qualitative variation because they occur during pregnancy but are absent in the sera of non-pregnant normal individuals. Placental alkaline phosphatase and oxytocinase are two well-known examples of pregnancy proteins. One of these is derived from the placenta the other is not (7 5)

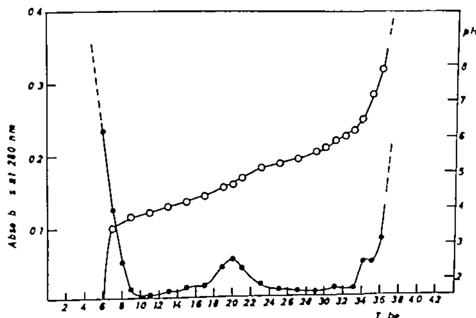


Fig 9 Isoelectric focusing performed on the purified "pregnancy zone protein in the pH-regio 3–6. ●—● Absorbance at 80 nm. ○—○ pH-gradient. 3 ml fractions were collected.

Acta Obstet Gynec Scand 52: 59–61 1973

GASTROINTESTINAL TRACT REACTION TO RADIATION THERAPY IN CANCER OF THE CERVIX MEASURED BY THE VITAMIN A ABSORPTION TEST

Per Bergsjö

From the Gynecological Department (Head: Professor Per Kolstad), The Norwegian Radium Hospital, Oslo and Norsk Hydro' Institute for Cancer Research (Head: Reidar Eker M.D.), Oslo, Norway

Abstract In an unselected group of 51 patients with carcinoma of the uterine cervix stage II vitamin A absorption tests were performed before treatment and at intervals during radiation treatment. All the patients received combination of external fractionated betatron treatments to external pelvic fields and local radium applications by modified Paris technique. The results show reduced absorption of vitamin A during radiotherapy with large individual variations but no apparent progression with increasing radiation dosage.

Gastrointestinal malfunction is a common side effect of radiation treatment for carcinoma of the uterine cervix, since the radiation also involves the intestines. The symptoms nausea, vomiting, abdominal cramps and diarrhoea are troublesome and may lower the general body resistance although they seldom require full discontinuation of the treatment. Fat absorption tests have been found useful in malabsorption syndromes (3) as a measure of the degree of intestinal absorption. Similarly in radiation-induced intestinal disorders Reeves et al. (6) have demonstrated reduced absorption rates of glyceroltrioleate and oleic acid. The fat soluble vitamin A may also be used as an indicator for intestinal fat absorption (4, 5). In the present study a vitamin A absorption test, slightly modified from previous descriptions (2, 3, 5) has been utilized. The purpose was to evaluate its usefulness as a clinical test in patients receiving radiotherapy for carcinoma of the cervix.

MATERIAL AND METHODS

Radiation treatment. This was a combination of local radium and external beams. The radium treatment consisted of two applications in succession, one intravaginal of 30 mg, and one intrauterine of 20 or 30 mg, each kept in place for 5 days. The external treatment was given with 31 or 33 MeV betatron X-rays to external pelvic fields, 314 cm² and 256 cm² in size respectively focus-skin distance 100 cm, 25 fractions with central shielding, each fraction of 200 R (Victoreen). Five to 6 fractions were given per week. Treatment started with 12 external fractions, followed by the two radium applications, and was finished with the last 13 external fractions. Further details of the treatment method and dose distributions have been given by Bergsjö & Kristiansen (1).

Vitamin A absorption test. The principle is determination of vitamin A in plasma 4 hours after intake of high test dose. The practical procedure was as follows.

At 8 a.m. a fasting heparinized blood sample was taken, following which the patient took the loading dose of 35 000 i.u. vitamin A palmitate in arachis oil (AFI-A dagvoeindom, A/S Farmaceutisk Industri, Oslo, Norway). At 8.30 the patient had an ordinary breakfast, which is necessary to promote normal gastrointestinal motility. At 12 o'clock (noon) a second blood sample was taken. Bed rest during the test hours was the rule.

This test was performed on 51 patients with carcinoma of the cervix stage II, before treatment and one to three times during the radiation treatment. The original plan was to perform the test at fixed times during treatment, but practical difficulties made this impossible.

In the laboratory each blood sample was centrifuged, and 5 ml heparinized plasma was shaken for 15 min with 10 ml absolute alcohol and 10 ml n-heptane. The heptane layer containing the vitamin A fraction, was then pipetted off and the optical density measured in a spectrophotometer at a wave length of 327 nm. This wave-length was found to give optimum density in a test sample of vitamin A in heptane, mixed with plasma. The concentration of vitamin A absorbed was then calculated according to the formula.

Optical density test sample − optical density pre-test sample × 4 200 = i.u. vitamin A per 100 ml plasma

ANTI-D-GLOBULIN BEHRING

300 MIKROGRAM

Bästa ekonomi!

Nu till marknadens lagsta priser

Enkel hantering!

Anti D Globulin Behring finns i ampuller med bruksfardig losning

Optimal dos!

Varje ampull innehåller 300 mikrogram Anti D

God hållbarhet!

Den basta hållbarheten for bruksfärdiga losningar 2 år





En pionjär inom immunbiologisk forskning

Svenska Hoechst AB Läkemedel
Box 42026 126 12 Stockholm 42
Tel 08 19 00 70

lity or through alterations of the bacterial flora. Wilson (7) has discussed the effects on intestinal cell-survival by different doses and fractionations of X-irradiation. If the premises for his calculations are correct the present scheme of external fractionated treatment may cause some disturbance of the intestinal villous cells, which may lead to impaired fat absorption. Altered motility resulting in vomiting and diarrhoea will also impair fat absorption. The relative contributions of these two mechanisms will probably fluctuate from patient to patient, which may partly explain the wide scatter of the results.

The vitamin A absorption test is clinically useful as a crude method during radiation therapy to detect cases of severe gastrointestinal malfunction. Consistently low values during treatment should be a warning sign that a temporary interruption of the treatment may become necessary.

ACKNOWLEDGEMENTS

The author was a Fellow of the Norwegian Cancer Society during the time of the study.

Dr. philos. Tore Sanner is gratefully acknowledged for his active cooperation in the plasma vitamin A analyses.

A/S Farmaceutisk Industri, Oslo, Norway kindly supplied the test samples of vitamin A (AFI A diagnosticum).

REFERENCES

1. Bergsjø, P. & Kristiansen, P. Treatment method and dose distribution in radiotherapy of carcinoma of the cervix. Acta Radiol (Ther.) *7* 181 192, 1968.
2. Gjone, E. & Forsdahl, A. A-vitaminbelastning ved malabsorpsjon. T Norske Lægeforen *82*. 1577–1578, 1962.
3. Ollendorff, P. A-vitamin-absorptionstest. I. Metode. Nord Med *61* 683–685, 1959.
4. Ollendorff, P. A-vitamin absorptionstest. II. Kliniske undersøgelser. Nord Med *61* 685–687 1959.
5. Paterson, J. C. S. & Wiggins, H. S. An estimation of plasma vitamin A and the vitamin A absorption test. J Clin Path *7* 56–60, 1954.
6. Reeves, R. J. Sanders, A. P. Sharpe, K. W. Thorne, W. A. & Isley J. K., Jr. Fat absorption from the human gastrointestinal tract in patients undergoing teletherapy. Amer J Roentgen *89* 122 126, 1963.
7. Wilson, C. W. Cell-survival data and the irradiation response of some normal tissues. Radiology *83* 120–130, 1964.

Submitted for publication Aug. 10 1972

Per Bergsjø
Department of Obstetrics and Gynecology
University of Bergen School of Medicine
5000 Bergen
Norway

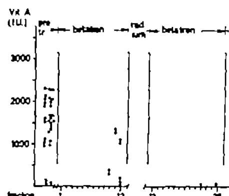


Fig 1 Results of the vitamin A absorption test.

Values below 1 000 I.u. vitamin A per 100 ml plasma were considered pathological (3).

The laboratory procedure described is a slight modification from previous methods of vitamin A determination for this test. Ultra-violet rays destroy vitamin A and determination has previously been based on the optical density difference between two samples of the same plasma, one irradiated by ultra-violet rays and a non-irradiated sample (2, 3 5) In the present study the laboratory work was simplified through omission of the ultra-violet lamp, since the patient's fasting pre-test plasma was used as a control sample.

RESULTS

The results are shown in Fig 1 There is a wide scatter for values both before and during treatment. It is seen that a number of the results before treatment are in the pathological range below 1 000 i.u. This may be due to negligence on the part of the patient to swallow the whole test dose of vitamin A to not taking her breakfast, to later vomiting, or to temporary gastrointestinal malfunction. Even though such breaks of the rule were sometimes reported the results have been included. Had they occurred during treatment the radiation would certainly have been blamed. Therefore the results may serve to demonstrate that not all pathological values found during treatment were necessarily due to the radiation.

Table I. *Results of the vitamin A absorption test*

	No. of obs.	Median values, and limits for 50 % of observations on each side of median		
		Lower	Median	Upper
Before treatment	39	1 038	1 520	2 079
Betatron fraction 6–12	21	302	1 063	1 466
Betatron fraction 13–19	15	926	1 037	1 186
Betatron fraction 20–25	27	514	940	1 735

Table II *Fraction of pathological vitamin A absorption tests*[a] *before and during different phases of treatment*

	Pathological tests/total no of tests	
Before treatment	9/39	23
Betatron fraction 6–12	10/21	48
Betatron fraction 13–19	6/15	40
Betatron fraction 20–25	14/27	52

[a] Defined as less than 1 000 I.u. vitamin A per 100 ml plasma following the test procedure described in text.

Fig. 1 leaves the impression that on average, the results are lower during than before treatment. This is borne out by Table I which shows the median values of the results before and during the different phases of the treatment. Table II shows the numbers of pathological values before and during treatment. The proportion of pathological tests is seen to be about doubled during treatment as compared to pretreatment values. It is also interesting to note that the malabsorption does not seem to be time or dose-dependent, the fraction of pathological values remaining fairly constant throughout the treatment period.

DISCUSSION

In the present series the vitamin A absorption test clearly showed a reduction of fat absorption during treatment. The maximum reduction of function at a dose of 2 000 R to the midline found by Reeves et al. (6), was not demonstrated. The treatment in this series, with radium interrupting the external beams, makes a dose response curve rather speculative.

The simplified laboratory procedure described makes the analysis very easy to perform For practical purposes close observations of the patients during test hours may be necessary for the correct interpretation.

Gastrointestinal symptoms during radiation treatment may be due to different causes such as a direct effect on intestinal epithelium, or indirectly via altered intestinal blood flow or motil-

Acta Obstet Gynec Scand 52, 63–67 1973

DETERMINATION OF PLASMA HUMAN CHORIONIC SOMATOMAMMOTROPHIN AND URINARY OESTRIOL IN DIABETIC PREGNANCIES

B Persson, N-O Lunell, M. L. Aubert, K. Carlström and J P Felber

From the Department of Obstetrics and Gynecology (Head. Professor Axel Ingelman-Sundberg), Sabbatsbergs Hospital, Karolinska Institute, the Department of Pediatrics (Head. Professor R Zetterström), St. Göran Hospital, Karolinska Institute Stockholm, Sweden, and the Department of Clinical Biochemistry (Head. Professor Jean-Pierre Felber), University Medical Clinic Lausanne Switzerland

Abstract Human chorionic somatomammotrophin (HCS) in plasma and oestriol excretion in urine were serially determined in 45 diabetic and 15 normal pregnancies. In the diabetic group there were three perinatal deaths. Mean HCS and oestriol values were not significantly influenced by factors such as maternal age, age at onset of diabetes, duration of diabetes or presence of retinopathy. Group mean HCS and oestriol values were not significantly different between diabetics and controls. Six diabetics had one or more HCS values below 4 µg ml. Four of them delivered small for date infants with one intra uterine and one neonatal death. In the former case death was preceded by a pronounced drop in oestriol and in the latter case oestriol values were low and fluctuating. Among the remaining women with low HCS values, three had normal and one had increasing oestriol values above the lower normal range. All women with HCS values above 4 µg ml had normal oestriol values and normal outcomes with one exception; this woman had low oestriol values and delivered a small for date infant with congenital rubella, who died as a result of hyaline membrane disease. It is concluded that determination of HCS in addition to determination of oestriol in pregnancies at risk would be of practical clinical value.

During recent years conflicting reports have been published concerning the value of plasma human chorionic somatomammotrophin (HCS) as a biochemical parameter to supervise at increased risk pregnancies (9 12, 13 17 19 21 22, 23 25). Reports on plasma HCS in diabetic pregnancies have shown higher than normal (3 17 19 20, 21) or normal values (1 12, 23, 24). There are few studies with simultaneous determinations of HCS and urinary excretion of oestriol (18, 22). Urinary oestriol excretion is considered a reliable index of foetal well-being (2, 5 7 8 10, 11 16). From a clinical point of view it seems important to demonstrate whether determination of HCS can give the same and/or additional information compared with urinary oestriol excretion. We have previously reported the clinical value of determination of urinary oestriol excretion in strictly controlled diabetic pregnancies (15). The present study deals with simultaneous determination of plasma HCS and urinary excretion of oestriol in 45 diabetic pregnancies.

MATERIAL AND METHODS

The series consisted of 3 groups of women; 15 controls, 32 women with insulin-requiring diabetes and 13 women with gestational diabetes. The women with gestational diabetes had a glucose disappearance rate of below 1.0% per min., reverting to normal after delivery. They were treated by diet alone. Ages, heights, pre-pregnancy weights, weight gains during pregnancy, gestational ages, birth weights and lengths of the infants and placental weights are given in Table I.

The diabetic women attended a special "mother" welfare clinic and were managed according to the principles given in an earlier report (15). White's classification (26) was used to group the diabetic pregnancies. The present series of insulin-requiring diabetics is a subgroup of 113 pregnancies in diabetic women with a total perinatal mortality of 4.4%.

Urinary oestriol excretion was determined according to Frandsen (6). HCS in plasma was analyzed according to Genazzani et al. (9). Oestriol and HCS was followed at least once weekly from the 32nd week of pregnancy.

RESULTS

The mean weekly HCS values in the control group and in the women with gestational and manifest



State University Groningen (The Netherlands)

In the Gynecological Department of the University Hospital Groningen (The Netherlands) will soon be a vacancy for a fully qualified

GYNECOLOGIST

being an experienced pelvic surgeon with scientific interest in oncological problems who intends to restrict his clinical work to the oncological patients and who will be able to give courses in the gynecological field

The appointment, a full time job will be in the grade as a lecturer

Information may be obtained from Prof Dr L.A. Joosse M D Chairman Department of Obstetrics and Gynecology Univer sity Hospital Groningen (The Netherlands) Oostersingel 59

Applications should be sent to the Chief of the Department of Personnel Matters Post Box 72 Groningen The Netherlands

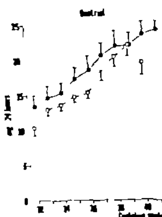


Fig. 2. Urinary oestriol excretion in insulin-treated diabetics and controls (mean ± S.E.M.). O Diabetes mellitus, ● control.

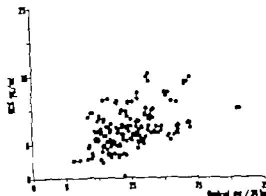


Fig. 3. Individual paired values of plasma HCS and urinary oestriol in insulin-treated diabetics.

in Table III. Four of these women gave birth to small for dates infants (SFD). Individual HCS and oestriol values from three of them (G. H., G. J., K. J.; see Table III) are given in Figs. 4 and 5 respectively. One of these mothers (G. H.) showed a fall in oestriol and the foetus died in utero. Another mother (K. J.) showed an irregular pattern of urinary oestriol excretion. She delivered a SFD infant with renal agenesis who died immediately after birth. The third woman (G. J.) had increasing oestriol values within the lower normal range. In no case was there a significant fall in oestriol without a simultaneous low HCS level. One diabetic patient (age 23 years, diabetic dura-

Table III. *Maternal and infant data on diabetic women with one or more HCS values below 4.0 µg/ml*

Case	HCS determinations Total no./no. < 4.0 (µg/ml)	Maternal data: Age (y)	Duration diabetes (y)	Age onset (y)	White group	Gest. age (weeks)	Delivery, special clinical features	Infant data: Sex	BW (g)	Length (cm)	Placental weight (g)	Course
G. H.	6/4	26	14	12	D	37	Vaginally induced	M	2 520	47	400	Intrauterine death; P.M. SFD[a], otherwise normal
M. M.	5/1	27	6	22	B	35	Vaginally induced	F	3 530	49	560	Uneventful
G. J.	17/15	33	17	16	C	39	Caesarean contracted pelvis	M	2 360	47	350	SFD, uneventful
B-M. J.	1/1	22	16	7	D	33	Caesarean preeclampsia	M	1 200	37	260	SFD, extrauterine asphyxia
C. J.	1/1	43	—	—	A	38	Caesarean imminent intrauterine asphyxia, contracted pelvis	F	3 200	50	520	Uneventful
K. J.	5	25	8	17	C	40	Vaginally induced	F	2 210	41	460	SFD, died 12 min after birth. P.M. renal agenesis

P.M. = post mortem examination. SFD = small for date infant.

5 – 732941

Table I *Maternal age height pre-pregnancy weight weight gain during pregnancy gestational age birth weight birth length and placental weight of the women and their newborn infants of the different groups*

Values given as mean ± standard error of mean (S.E.M)

	No.	Age (y)	Weight (kg)	Height (cm)	Weight gain (kg)	Gest. age (weeks)	Birth weight (g)	Birth length (cm)	Placental weight (g)
Mean		27 6	55.8	165 1	14 1	40.6	3 51	50.9	459
Controls	15								
S.E.M		0.7	1 4	1.5	1 4	0.3	156	0.5	17
Mean		26.1	59 6	166.0	9.3	38.3	3 276	49.2	543
Diabetics	32								
S.E.M		0.7	2.0	1 1	0.5	0.3	155	0.6	27
Mean		28.0	64.[illegible]	163.2	8.6	39.9	3 758	52.0	533
Gest. Diabetics	13								
S.E.M		1 7	3 8	2.0	0.8	0.2	127	0.5	37

diabetes are given in Table II and Fig. 1 In all groups there was a steady increase of the HCS values with gestational age. There were no statistical differences between the three groups when values from corresponding weeks were compared. When weekly HCS values in diabetic women belonging to White s classes B and C ($n = 22$) were grouped together and compared with those of women in class D ($n = 10$) no significant statistical differences were found. Nor were any statistical differences in HCS values observed when the following subgroups were compared. Woman s age 18–26 years ($n = 20$) versus 27–37 years ($n = 12$) duration of diabetes less than 10 years ($n = 17$) versus more than 10 years ($n = 15$) onset of diabetes before 10 years of age ($n = 10$) versus after 10 years of age ($n = 22$) or retinopathy ($n = 9$) versus no retinopathy ($n = 23$)

Table II. *Mean weekly (±S E.M) HCS values in control women and women with gestational and manifest diabetes*

Pregnancy week	32	33	34	35	36	37	38	39	40
Controls, $n = 15$									
Mean	6.6	6.2	7.2	7.0	7.3	8.6	9.0	8.9	10.0
S.E.M.	0.9	0.8	0.7	0.9	1.0	1.0	1.2	1 1	1 4
Gest. diabetes, $n = 13$									
Mean							7.0	9.3	7 4
S.E.M							1 1	1 0	0 6
Manifest diabetes, $n = 32$									
Mean	6.6	7 0	7 8	8.3	9.3	9 1	8.6	8.9	6 6
S.E.M.	1.5	0 6	0.7	0.7	0.8	0.8	0.7	1 4	1 1

The mean weekly urinary oestriol excretion in the control group and in the women with gestational and manifest diabetes showed a steady increase with gestational age (Fig. 2) As with HCS, there were no statistical differences between corresponding weekly values in the three groups. There were no significant differences between the mean oestriol values of the above mentioned subgroups of diabetic women. Regression analyses of individual paired values of HCS and oestriol showed no correlation in the control group but a significant relationship was found in the gestational diabetic group ($r = 0.37$ $p < 0.02$) and in the insulin treated groups ($r = 0.34$ $p < 0.001$ Fig. 3).

One woman with gestational and 5 women with overt diabetes had HCS values below 4 µg/ml. Maternal and infant data for this group are given

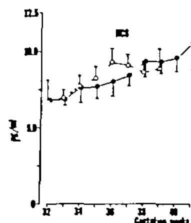


Fig 1 Plasma HCS values in insulin-treated diabetics and controls (mean ± S.E.M.). ○ Diabetes mellitus, ● control.

values which were decreasing slightly with gestational age, indicating some disturbance of the foeto-placental function. Lower HCS values could have been expected since the infant was severely intra-uterine growth retarded, probably explained by the congenital rubella infection. The normal HCS values and the low oestriol values could possibly indicate a more severe affection of the foetus than of the placenta. This observation further supports the above suggestion of the clinical value of simultaneous determinations of HCS and oestriol. Further studies are necessary to demonstrate whether simultaneous assays of oestriol and HCS could differentiate between different causes of intra-uterine growth retardation, i.e. between foetal viral infections, notably rubella, foetal chromosome aberrations—both of which probably delay DNA synthesis (4)—and e.g. maternal hypertension, or pre-eclampsia on the other side.

In those mothers with persistently low HCS concentrations the urinary oestriol excretion in one case showed a pronounced fall, in another a low irregular pattern and in a third increasing values within the lower normal range (Figs. 4 and 5). From this limited numbers of observations it seems as if low HCS values could predict foetal danger earlier than the urinary oestriol pattern. On the other hand low HCS values do not give information regarding the proper timing of delivery. In conclusion, determination of HCS in addition to the determination of urinary oestriol excretion in pregnancies at risk could be of practical clinical value.

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish Diabetes Association, the Swedish Medical Research Council (project 13P 3268) and by the Swiss National Fund for Scientific Research (request no. 3.3.44.70).

REFERENCES

1. Beck, P., Parker, M. L. & Daughaday, W. H.: J Clin Endocrin 25 1457, 1965.
2. Bjoro, K.: Acta Endocrin (Kbh), Suppl. 161, 1972.
3. Cohen, M., Haour, F. & Bertrand, J.: Gynecol Obstet (Paris) 69 197, 1970.
4. Editorial: Lancet 2 856, 1971.
5. Fransson, V. & Stakeman, G.: Acta Endocrin (Kbh) 44 183, 1963.
6. Fransson, V.: The excretion of oestriol in normal human pregnancy. Munksgaard, Copenhagen, 1963.
7. Fuchs, M.: Acta Obstet Gynec Scand 41 370, 1962.
8. Galbraith, R. S., Low, J. A. & Boston, R. W.: Amer J Obstet Gynec 106, 352, 1970.
9. Genazzani, A. R., Aubert, M. L., Casoli, R., Fioretti, P. & Felber, J.-P.: Lancet 2: 1385, 1969.
10. Greene, J. W. & Touchstone, J. C.: Amer J Obstet Gynec 85 1, 1963.
11. Heys, R. F., Scott, J. S., Oakey, R. E. & Stitch, S. R.: Obstet Gynec 33 390, 1969.
12. Josimovich, J. B., Kosor, B., Boccella, L., Mintz, D. H. & Hutchinson, D. L.: Obstet Gynec 36 244, 1970.
13. Keller, P. J., Baertschi, U., Bader, P., Gerber, C., Schmid, J., Soltermann, R. & Kopper, E.: Lancet 2 729, 1971.
14. Letchworth, A. T. & Chard, T.: Lancet 1 704, 1972.
15. Persson, B., Lunell, N.-O., Carlström, K. & Paro-hjelm, M.: Acta Obstet Gynec Scand 49 379, 1970.
16. Rivlin, M. E., Mestman, J. H., Hall, T. D., Weaver, C. P. & Anderson, G. W.: Amer J Obstet Gynec 106 875, 1970.
17. Saxena, N. A., Bradbury, J. & Goplerud, C.: Amer J Obstet Gynec 104 781, 1969.
18. Saxena, N. A., Gallagher, H. S., McRoberts, W. A. & Faris, A. M.: Amer J Obstet Gynec 109 63, 1971.
19. Saxena, B. N., Emerson, K. & Selenkow, H. A.: New Engl J Med 281 225, 1969.
20. Selenkow, H. A., Varma, K., Younger, D., White, P. & Emerson, K.: Diabetes 20 696, 1971.
21. Singer, W., Desjardins, P. & Friesen, H. G.: Obstet Gynec 36 222, 1970.
22. Spellacy, W. H., Teoh, E. S., Buhi, W. C., Birk, S. A. & McCreary, S. A.: Amer J Obstet Gynec 109 588, 1971.
23. Sposm, J. & Jaskisch, H.: Acta Endocrin (Kbh) 68 401, 1971.
24. Teoh, E. S., Spellacy, W. N. & Buhi, W. C.: J Obstet Gynec Brit Comm 78 673, 1971.
25. Varma, K., Driscoll, S. G., Emerson, K. & Selenkow, H. A.: Obstet Gynec 38 487, 1971.
26. White, P.: Med Clin N Amer 49 1015, 1965.

Submitted for publication Aug. 11, 1972

Bengt Persson
Department of Pediatrics
St. Görans Hospital
Box 12500
S-112 81 Stockholm
Sweden

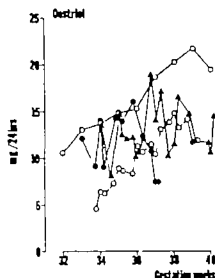


Fig 4 Urinary oestriol excretion in 3 diabetic women with HCS values below 4 μg/ml. ●—● G. H., ○—○ G J ▲—▲ K. J ○—○ mean diabetics.

tion 14 years, White group C) with normal HCS values of 6 7 7 9 μg/ml in week 34 and 35 respectively and with oestriol values of 8 0 8 0 7 6 6 6 in weeks 33 34 35 and 36 respectively developed abruptio placentae in the 36th week. She was delivered by cesarean section. The infant was severely asphyxiated at birth and died at 6 hours as a result of respiratory distress syndrome Postmortem examination showed an intrauterine growth retarded infant (birth weight 1 600 g length 44 cm) with malformations of the left hand (oligodactyly + syndactyly) hyaline membranes and histological changes in lungs and skeleton compatible with congenital rubella. Changes in maternal haemaglutination inhibiting antibody

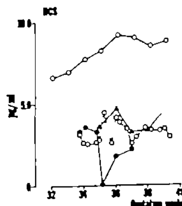


Fig 5 Three diabetic women with plasma HCS values below 4 μg/ml. ●—● G H. ○- -○ G. J., ▲—▲ K. J ○—○ mean diabetics.

titers during pregnancy confirmed a subclinical infection with rubella, which probably occurred before the 15th week of gestation.

DISCUSSION

The present findings of normal group mean plasma HCS values during the last trimeter in diabetic pregnancies are in accordance with some previous reports (12, 23 24) Neither mean HCS values nor mean urinary excretion of oestriol in the diabetic women were significantly influenced by factors such a maternal age onset of diabetes, duration of diabetes or presence of retinopathy

Th individual paired values for HCS and oestriol showed a wide scatter (Fig. 3) which increased with gestational age. Although these values were significantly interrelated in the two diabetic groups, th correlation coefficients were low ($r=$ 0 34 and 0 37) indicating a weak biological relationship. Spellacy et al (22) found a correlation between HCS and oestriol significant only up to the 35th week in normal pregnancies ($r=0.77$). No correlation was found among diabetic pregnancies below the 35th week of gestation. These observations and the greater clinical importance of values obtained during the latter weeks of pregnancy support the suggestion of Spellacy et al. (22) that both HCS and oestriol determination would give more information than either test alone. Based on a great number of plasma HCS determinations in pregnancies at risk Spellacy et al. (22) described a foetal danger zone defined as HCS values below 4 μg/ml during the last 10 weeks of pregnancy In the present study six diabetic women had one or more HCS values below 4 μg/ml during the last trimester Four of these women delivered small for dates infants, two of whom died perinatally The remaining two mothers delivered normal infants. However one of these mothers had only one HCS determination and the other had one of 5 which was below 4 μg/ml. These results are thus in accordance with previous reports (14 22) and indicate that low HCS values could predict foetal danger and also that the risk of foetal distress in labor or of neonatal asphyxia increases with an increasing number of serial observations of low HCS values. All diabetic patients with HCS values above 4 μg/ml had normal outcomes of their pregnancies with one exception This woman had low oestriol

Acta Obstet Gynec Scand 52, 69–76, 1973

THE URETEROCALYCEAL SYSTEM IN NORMAL PREGNANCY

A Study using Isotope Renography and Intravenous Pyelography

M. M. Fayad, A. F. Youssef, M. Zahran, M. Kamel and M. Badr

From the Department of Obstetrics and Gynaecology (Head, Professor A. F. Youssef) and the Department of Urology (Head, Professor M. Badr), Kasr-el-Aini Hospital, Cairo, Egypt UAR

Abstract. Sixty normal pregnant women were investigated by isotope renography and by intravenous pyelography. In 92.3% of patients in the third trimester of pregnancy both methods revealed the presence of ureterocalyceal obstruction. Renography was found to be the more sensitive method. Strong evidence is presented to suggest that mechanical pressure from the pregnant uterus is the essential factor in the causation of the changes observed.

The original observation of Cruveilhier (6), based on autopsy findings, that the ureter and renal calyces are frequently dilated during pregnancy were confirmed by many subsequent authors who used radiographic techniques. Traut & McLane (23) for example, found that this dilatation was present in over 80% of women X-rayed in the second half of pregnancy. The literature on this topic was reviewed by Crabtree (5).

More recently however, Kremling (13, 14) stated that his pyelographic investigations of healthy pregnant women did not show any changes in the ureters and renal calyces; atony and dilatation were only found in patients with urinary tract infection or other urological pathology either before or *during* pregnancy. Burger (4) expressed the same views and concluded that in pregnancy it was not the atony or dilatation of the ureter and calyces which predisposed to infection but the infection which predisposed to atony.

The advent of the technique of isotope renography (22) and the demonstration that it can be safely used in pregnancy (24), tempted us to re-examine the true incidence of changes in the upper urinary tract in normal pregnant women. Moreover, the numerous authors who maintained that ureterocalyceal dilatation was a frequent accompaniment of normal pregnancy disagreed as to whether this dilatation was brought about by hormonal effects (12, 9, 18) or by mechanical factors (8, 7, 17). We have, therefore, laid emphasis on examining the possible role of mechanical obstruction by the pregnant uterus in the causation of changes in the ureterocalyceal system.

MATERIAL AND METHODS

Sixty normal pregnant women, aged 18 to 38 years, were investigated. Forty-eight were multigravidae and 12 were primigravidae. Three were in the first, 5 in the second and 52 in the third trimester of pregnancy. The presentation, ascertained in 54 patients, was cephalic in 51, transverse in one, complete breech in one and frank breech in one patient. Patients with toxaemia, hypertension or history of urological disease were not included.

Renography was carried out with Hippuran I^{131} as tracer in a dose of 1 μCi/5 kg body weight. The Picker dual detector machine with two scintillator probes and double recording apparatus at a rate of 12 inches (304.8 mm) per hour was used. The sensitivity was K_{4} and the time constant 10 seconds. Renography was first performed with the patient sitting upright and then repeated 24 hours later with the patient in the genupectoral position.

The interpretation of the renogram was discussed by Baird et al. (2) and Rudolph & Max (24). In the normal renogram the curve shows three segments: an initial steep almost perpendicular ascent or the "vascular phase"; this is followed by a slower rise or the "secretory phase"; finally the curve falls and this is the "excretory phase". Obstruction results in an abnormal appearance of the excretory phase and it is this phase of the renogram which has been investigated in the present study. Depending on the degree of obstruction the excretory phase tracing may descend at an abnormally slow rate, be horizontal or even rise.

Except for the three in the first trimester of pregnancy all patients were also examined by intravenous pyelography. A double dose of Vasurix was injected and

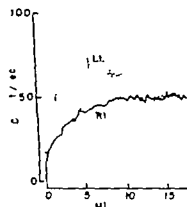


Fig. 4. Renogram showing bilateral ureteric obstruction, more marked on the right than on the left.

The effect of the presentation of the fetus is shown in Table III.

One patient with a complete breech presentation and one with the fetus in a transverse lie had normal renographic and pyelographic findings. In one patient with a deeply engaged frank breech and in the large majority of patients with a fetus presenting by the head evidence of ureteric obstruction was obtained (Figs. 6 to 9). Obstruction was more common in primigravidae than in multigravidae (Table IV) and in patients with strong rather than lax abdominal musculature (Table V and Figs. 2, 3, 10 and 11).

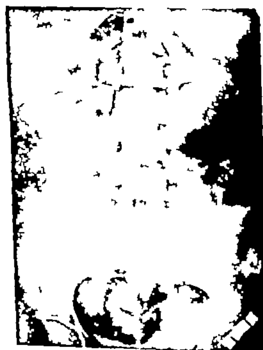


Fig. 5. Intravenous pyelogram showing obstruction, slight dilatation and tortuosity of the right ureter and normal left ureter.

Table II. *Isotope renography and intravenous pyelography during pregnancy. Side affected by ureteric obstruction (patients in first trimester of pregnancy excluded)*

Result	Renography (57 patients, sitting)	I. pyelography (57 patients, supine)
No obstruction	8	8
Bilateral obstruction	46	44
both sides equal	10	10
more marked on right	36	34
more marked on left	0	0
Right side only obstructed	3	5
Left side only obstructed	0	0

The important effect of posture in the causation of ureterocalyceal obstruction in normal pregnant women is shown in Table VI. In all but 2 patients re-examined in the genupectoral position both renography and pyelography showed marked relief of ureteric obstruction (Figs. 2, 9, 12 and 13).

In all patients showing evidence of ureterocalyceal obstruction who were re-examined 24 hours after delivery, evidence of partial relief was noted. All such patients re-examined 1 or 2 weeks postpartum had normal renograms and pyelograms (Table VII and Figs. 4, 5, 14 and 15).

DISCUSSION

There are few published reports on the use of isotope renography in pregnant patients. Laakso

Table III. *Isotope renography and intravenous pyelography during pregnancy. Effect of presentation of fetus on results*

Presentation or Lie (no.)	Renography (54 patients, sitting)	I. pyelography (54 patients, supine)
Cephalic (51)	3 Normal 48 Obstruction	3 Normal 48 Obstruction
Frank breech (1)	1 Obstruction	1 Obstruction
Complete breech (1)	1 Normal	1 Normal
Transverse (1)	1 Normal	1 Normal

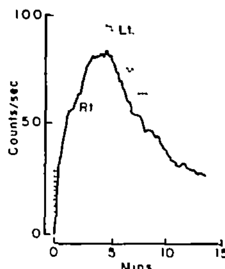


Fig 1 Normal renogram from a primigravida, 1 weeks pregnant.



Fig 3 Intra enous pyelogram showing bilateral obstruction from a primigravida, 36 weeks pregnant.

two exposures were made the first after 10 minutes with the patient in the supine position and the second 15 minutes later with the patient in the genupectoral position.

Intravenous pyelography in pregnancy has been carried out by many previous investigators. The safety of the method has been discussed by Kremling (13) and by Burger (4). In most of our patients in whom this method was used, radiographic examination was indicated for other reasons, chiefly to obtain information about the foetus, two exposures only were made in each case In all cases the consent of the patient to using intravenous pyelography was obtained.

Some of the patients showing evidence of obstruction were followed up postpartum. Renography in the sitting upright position and pyelography in the supine position were carried out 4 hours after delivery in 4 patients, 1 week after delivery in 1 patients, and weeks after delivery in 5 patients.

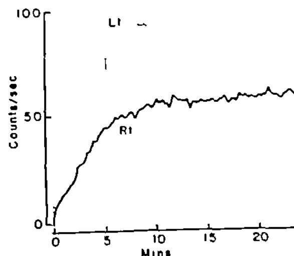


Fig 2 Renogram showing bilateral obstruction from primigravida, 32 weeks pregnant.

RESULTS

The effect of the duration of pregnancy on the renographic and pyelographic findings is shown in Table I. The ureterocalyceal system was normal in all patients in the first trimester of pregnancy (Fig. 1) while obstruction was noted in the large majority of patients in the third trimester of pregnancy (Figs. 2 and 3)

In most patients showing obstruction the abnormality was bilateral. In the majority it was more marked on the right than on the left (Table II and Fig. 4) In very few patients did the right side only show evidence of obstruction (Fig. 5). In no patient was obstruction confined to or more marked on the left side.

Table I *Isotope renography and intravenous pyelography during pregnancy Effect of duration of pregnancy on results*

Duration of pregnancy	Renography (60 patients, sitting upright)	I pyelography (57 patients, supine)
First trimester	3 Normal	Not done
Second trimester	4 Normal 1 Mild obstruction	4 Normal 1 Mild obstruction
Third trimester	4 Normal 48 Obstruction	4 Normal 48 Obstruction

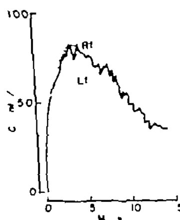


Fig. 10 Normal renogram from multigravida (38 weeks pregnant) with lax abdominal muscles.

found that obstruction was partially relieved when the patient changed from the supine to the lateral position.

In these publications the effects of parity of the tone of abdominal musculature and of the presentation of the fetus were not investigated or



Fig. 11 Normal intravenous pyelogram from multigravida (38 weeks pregnant) with lax abdominal muscles.

Table IV *Isotope renography and intravenous pyelography during pregnancy. Effect of parity on results*

Parity (no. of pats.)	Renography (57 patients)	I. pyelography (57 patients)
Primigravida (12)	1 Normal	1 Normal
	11 Obstruction	11 Obstruction
Multigravida (45)	6 Normal	7 Normal
	39 Obstruction	38 Obstruction

correlated with findings on intravenous pyelography.

Our observation that 48 of 52 normal pregnant patients in the third trimester (92.3%) showed renographic and pyelographic evidence of ureterocalyceal obstruction supports the classical views stated by Kretschmer et al. (15), Baird (1), Hundley et al. (11) and Traut & McLane (23) and disagrees with the view expressed by some recent writers (4, 14) that such obstruction does not, as a rule, occur in healthy pregnant women. Our experience (Tables I to VII) indicates that intravenous pyelography with the double dose technique yields results very similar to those obtained by isotope renography. The latter method, however, appeared to be the more sensitive, for in every instance when intravenous pyelography revealed obstruction renography showed delay in the excretory phase, whereas in two cases of mild left ureteric obstruction demonstrated by renography the pyelogram was normal (Table II).

Our results provide strong evidence that mechanical pressure by the pregnant uterus is by far the most important factor in the causation of ureterocalyceal obstruction in normal pregnant women. In favour of this viewpoint are the increased incidence and degree of obstruction with the advance of pregnancy, the more frequent and

Table V *Isotope renography and intravenous pyelography during pregnancy. Effect of abdominal musculature on results*

Muscle tone (no. of pats.)	Renography (57 patients)	I. pyelography (57 patients)
Strong (16)	1 Normal	1 Normal
	15 Obstruction	15 Obstruction
Moderate (38)	4 Normal	4 Normal
	34 Obstruction	34 Obstruction
Lax (3)	3 Normal	3 Normal

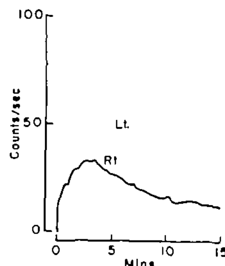


Fig 6 Normal renogram with the fetus lying transversely

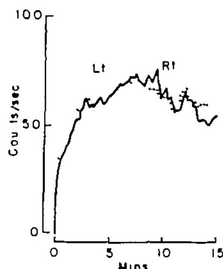


Fig 8 Renogram showing obstruction with the fetus presenting as a frank breech.

(16) by comparing secretory phase measurements, found that renal function was slightly reduced ante partum in patients with toxaemia and hyper tension and that there was significant improvement after delivery Rudolph & Wax (20) who studied the excretory phase in normal pregnant women found evidence of obstruction which was more marked on the right than on the left side in the last trimester of pregnancy and which disappeared 48 hours after delivery They did not ex amine the effect of posture on the renogram. Baird et al. (2) reported similar observations and



Fig 7 Normal intravenous pyelogram with the fetus presenting as a complete breech.



Fig 9 Intravenous pyelogram showing ureteric obstruction with the fetus presenting as a frank breech.

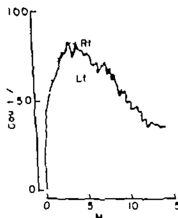


Fig. 10. Normal renogram from multigravida (3[illegible] weeks pregnant) with lax abdominal muscles.

found that obstruction was partially relieved when the patient changed from the supine to the lateral position.

In these publications the effects of parity of the tone of abdominal musculature and of the presentation of the fetus were not investigated or



Fig. 11. Normal intravenous pyelogram from multigravida (34 weeks pregnant) with lax abdominal muscles.

Table IV. *Isotope renography and intravenous pyelography during pregnancy. Effect of parity on results*

Parity (no. of pats.)	Renography (57 patients)	I.[v.] pyelography (57 patients)
Primigravida (12)	1 Normal	1 Normal
	11 Obstruction	11 Obstruction
Multigravida (45)	6 Normal	7 Normal
	39 Obstruction	38 Obstruction

correlated with findings on intravenous pyelography.

Our observation that 48 of 52 normal pregnant patients in the third trimester (92.3 %) showed renographic and pyelographic evidence of ureterocalyceal obstruction supports the classical views stated by Kretschmer et al. (15), Baird (1), Hundley et al. (11) and Traut & McLane (23) and disagrees with the view expressed by some recent writers (4, 14) that such obstruction does not, as a rule, occur in healthy pregnant women. Our exp[e]rience (Tables I to VII) indicates that intravenous pyelography with the double dose technique yields results very similar to those obtained by isotope renography. The latter method, however, appeared to be the more sensitive, for in every instance when intravenous pyelography revealed obstruction renography showed delay in the excretory phase, whereas in tw[o] cases of mild left ureteric obstruction demonstrated by renography the py[e]logram was normal (Table II).

Our results provide strong evidence that mechanical pressure by the pregnant uterus is by far the most important factor in the causation of ureterocalyceal obstruction in normal pregnant women. In favour o[f] this viewpoint are the increased incidence and degree of obstruction with the advance of pregnancy, the more frequent and

Table V. *Isotope renography and intravenous pyelography during pregnancy. Effect of abdominal musculature on results*

Muscle tone (no. of pats.)	Renography (57 patients)	I.[v.] pyelography (57 patients)
Strong (16)	1 Normal	1 Normal
	15 Obstruction	15 Obstruction
Moderate (38)	4 Normal	4 Normal
	34 Obstruction	34 Obstruction
Lax (3)	3 Normal	3 Normal

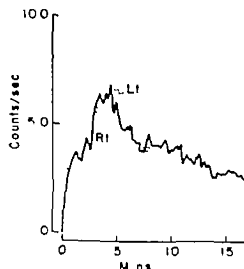


Fig 12 Renogram from the same patient as in Fig. 4 when re-examined in the genupectoral position. Note partial relief of ureteric obstruction.

often more pronounced obstruction on the right than on the left side the effect of presentation of the fetus, the effect of parity and of the tone of the abdominal musculature the marked relief seen in the large majority of cases when re



Fig 13 Intravenous pyelogram from the same patient as in Fig. 9 when re-examined in the genupectoral position Note partial relief of ureteric obstruction.

Table VI *Isotope renography and intravenous pyelography during pregnancy Effect of posture on results in 49 patients with evidence of bilateral or unilateral ureteric obstruction*

	Renography	I v pyelography
Result when patient re-examined in genupectoral position	47 Relief 2[a] No relief	47 Relief 2[a] No relief

[a] Both were primigravidae with strong abdominal muscles and a fetus presenting by the vertex.

examined in the genupectoral position, the considerable amelioration observed 24 hours after delivery and the complete return to normal at the end of the first week post partum. Certain reports in the literature are of interest in this regard. Mengert (19) failed to find ureterocalyceal dilatation in eight different species of quadrupeds in which posture obviated pressure of the uterus upon the ureters. Everett (8) followed with repeated pyelograms a patient with a horse-shoe kidney in whom the ureters did not extend above the pelvic brim and found that there was no evidence of ureterocalyceal dilatation as a result of pregnancy Gerbie & Flanagan (10), using isotope renography found evidence of ureterocalyceal obstruction in some patients with pelvic inflammatory disease endometriosis or large uterine fibromyomata

Our pyelographic finding that ureteric dilata

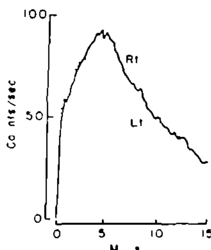


Fig 14 Renogram from the same patient as in Fig. 4 at 1 week after delivery Note complete relief of ureteric obstruction.

tion always began at the level of the pelvic brim likewise supports the mechanical theory and is in agreement with the observations of many previous authors including Everett (8) and Donald (7). Donald believes that the ureter at the pelvic brim is more exposed *to pressure on the right side* because of the protection given the left ureter by the sigmoid colon and its mesentery and because of the presence of frequent dextrorotation of the uterus. Recently Bellina et al. (3) suggested that right ureteric dilatation in pregnancy might be explained by the anatomical relationship between the right ovarian venous plexus and the right ureter. In our view this can only be considered to enhance the all-important effect of the gravid uterus.

However there is no definite evidence that hormonally conditioned ureteric atony does not play a role in predisposing to the changes observed in the upper urinary tract in normal pregnancy. Cystometric studies in our Department showed that the tone of the bladder is much decreased and its capacity much increased in normal pregnancy (25, 26). Traut & McLane (23) and Hundley



Fig. 1. Intravenous pyelogram from the same patient as in Fig. 3, at 1 week after delivery. Note complete relief of ureteric obstruction.

Table VII. *Isotope renography and intravenous pyelography during and after pregnancy. Results of re-examination post partum*

Time post partum (no. of pats.)	Renography	Intravenous pyelography
24 hours (4)	4 Partial relief	4 Partial relief
One week (12)	12 Normal	12 Normal
Two weeks (5)	5 Normal	5 Normal

et al. (12) maintained that ureteric tone and contractility diminish as pregnancy advances and believed that this was dependent on hormonal secretion especially progesterone. More recently Sala & Rubi (21) criticized the technique used by these authors and found that the intensity, frequency and tonus of ureteric contractions apparently did not change throughout pregnancy. It may therefore be concluded that hormonally caused softening, atony or diminished contractility of the ureter, if such does exist in normal pregnancy, at best contribute to the changes in the upper urinary tract observed in this study but are by themselves unable to explain the results we obtained.

REFERENCES

1. Baird, D. J Obstet Gynaec Brit Emp 42, 577, 1935.
2. Baird, D. L., Garrett, P. W. & Doug, A. Am J Obstet Gynec 95, 597, 1966.
3. Bellina, J. H., Dougherty, C. M. & Mickal, A. Am J Obstet Gynec *108*, 356, 1970.
4. Barger, K. Gynaecologia *141*, 113, 1956.
5. Crabtree, E. G. Urological Diseases of Pregnancy. Little, Brown & Co. Boston, 1942.
6. Cruveilhier, J. Descriptive Anatomy. Whitaker & Co., London, 1841.
7. Donald, I. British Obstetric and Gynaecological Practice: Obstetrics (ed. *Eardley Holland*). Heinemann, London, 1955.
8. Everett, H. S. Gynecological and Obstetrical Urology. Williams & Wilkins Co., *Baltimore*, 1947.
9. Fainstat, L. Am J Obstet Gynec *87*, 486, 1963.
10. Gerbie, A. B. & Flanagan, C. L. Am J Obstet Gynec *84*, 1838, 1962.
11. Hundley, J. M., Walton, H. J., *Hibbitts, J. T.*, *Siegel*, L. A. & Brack, C. B. Am J Obstet Gynec *30*, 625, 1935.
12. Hundley, J. M., Diehl, W. K. & Diggs, E. S. Am J Obstet Gynec *44*, 858, 1942.
13. Kraemling, H. Zbl Gynäk *76*, 2171, 1954.
14. Kraemling, H. *I*: Gynecological Urology (ed. A. F. Youssef). C. C. Thomas, Springfield, Ill., 1960.
15. Kretschmer, H. L., Heaney, N. S. & Ockuly, E. A. *J Am Med Ass 101*, 2025, 1933.

16. Laakso, L. Acta Obstet Gynec Scand 46 Suppl. 5 1967
17 La Pata, R. E., McElin, T W & Adelson, B. H. Am J Obstet Gynec 106. 941 1970.
18 McNellis, D Am J Obstet Gynec 101 265 1968.
19 Mengert, W F Am J Obstet Gynec 27 544 1934
20. Rudolph, J H. & Wax, S. H. Obstet Gynec (NY) 30. 346, 1967
21 Sala, N L. & Rubi, R. A., Am J Obstet Gynec 9 918, 1965
22. Taphn, G Meredith, O Kade H. & Winter C., J Lab Clin Med 48 886 1956.
23 Traut, H F & McLane C. M Surg Gynec. Obstet 6 65 1936.
24. Wax, S. H. & Rodolph, J H. Obstet Gynec (NY) 30 381 1967
25 Yousef, A. F. Obstet Gynec (NY) 13 61 1959
26. Yousef, A. F In Gynecological Urology C. C. Thomas, Springfield, Ill 1960.

Submitted for publication August 19 1972

Abdel Fattah Yousef
2, Sherif Pasha Street, Apt 43
Cairo
Egypt
UAR

Acta Obstet Gynec Scand 52: 77–81, 1973

EXPERIENCE WITH SHIRODKAR'S OPERATION AND POSTOPERATIVE ALCOHOL TREATMENT[1]

Niels H. Lauersen and Fritz Fuchs

From the Department of Obstetrics and Gynecology (Head: Prof. Fritz Fuchs) The New York Hospital Cornell Medical Center New York, USA

Abstract During the period 1960–1970, the post-conceptional cerclage of the cervix as first described by Shirodkar was used in 143 patients with cervical incompetence. The majority of the patients received heavy sedation with sodium amytal and demerol postoperatively. A smaller group was treated prophylactically with alcohol intravenously and another small group was treated with alcohol after failure of the sedation regime to prevent uterine activity. The success rate was the same in groups 1 and 2 (83 %); in the cases where alcohol was given after the occurrence of uterine contractions, the success rate was 71 %.

The year of the death of Dr V. N. Shirodkar of India, 1971, seems to be a pertinent time to review the results of the operation which Dr Shirodkar described in 1954 (21).

During the period 1960–1970, a post-conceptional Shirodkar cerclage operation was done in 143 patients. Of these 106 patients were treated with heavy sedation and bed rest postoperatively. When Fuchs et al. (5) first introduced the use of intravenous alcohol for inhibition of threatened premature labor, we began using intravenous alcohol prophylactically in the immediate postoperative period (23 instances) and also used intravenous alcohol in cases (14) where sedation treatment failed and premature labor occurred.

MATERIAL

Retrospective studies of 143 patients who underwent post-conceptional Shirodkar cerclage operations at the New York Hospital-Cornell Medical Center were carried out. The clinic patients were, in the earlier years of the study, followed in the Habitual Abortion Clinic, now they are followed in the High Risk Clinic. Patients with history of habitual abortions, when first seen in the antepartum clinic, were referred to the Special Clinic. Here they underwent weekly internal examination with visualization of the cervix. If any dilatation of the internal os occurred during the mid-trimester, the patients were usually considered candidates for post-conceptional Shirodkar cerclage. In general, hysterosalpingogram was obtained if a patient had three or more late abortions. If the X-ray confirmed an incompetent internal os, the patient was permitted to become pregnant and a post-conceptional Shirodkar procedure was performed. The operation was usually done around 14–16 weeks of gestation. A post-conceptional Shirodkar procedure was preferred to a pre-conceptional Shirodkar procedure or Lash operation.

Most of the private patients were followed according to the same protocol as the clinic patients. However, the decision to perform the operation was left to the individual attending physician. Several of the cases, in the earlier years of the study were referred when already pregnant through other physicians with a diagnosis of an incompetent cervical os made elsewhere.

The chart review does not permit an evaluation of criteria used by individual members of the attending staff. No patients had uterine contractions at the time of surgery.

The outcome of the previous pregnancies in the 143 patients are shown in Table 1.

PROCEDURE

The operation which has been carried out at The New York Hospital since 1958, is a modification of Shirodkar's technique described by Barter et al. (1, 2). Shirodkar used fascia lata strip rather than a suture. Otherwise the operation is grossly the same.

After proper preparation of the vagina, a curved incision is made anteriorly at the junction of the cervix and the bladder. With blunt dissection, the bladder is pushed upward to the level of the internal os. Posteriorly a similar incision is carried out to expose the cervix. A mersilene tape is then placed at the level of the internal os with the help of aneurysm needles, and the suture is

[1] Presented in part at the District II meeting of the American College of Obstetricians and Gynecologists, Aruba, October 1971.

Table I *Post-conceptional Shirodkar procedures Outcome of previous pregnancies in 143 patients*

No previous abortion		18
Admitted with cervical dilation >4 cm	12	
Previous early abortions		14
1 induced abortion	2	
2 spontaneous abortions	6	
3 or more spontaneous abortions	6	
Previous late abortions and/or premature delivery		111
1 mid-trimester abortion	5	
2 mid-trimester abortions	9	
3–5 late abortions	6	
6–7 late abortions	4	
Premature delivery with neonatal death	12	
Premature delivery with live birth	4	
Premature delivery with neonatal death and 1 mid-trimester abortion	19	
Premature delivery with live births and 1 mid-trimester abortion	10	
Premature del very with neonatal death and 2 mid-trimester abortions	8	
Premature delivery with live births and 1 mid-trimester abortion	1	
Premature delivery with neonatal death and 3 or more mid trimester abortions	16	
Premature delivery with live births and 3 or more mid-trimester abortions	17	
Total		143

tied with a number 4 Hegar dilator in the cervix. The vaginal mucosa is then closed over the suture. In our earlier cases, two nylon sutures were used instead of merulene.

The Shirodkar procedure is easier and more safely done when the cervix is 2 cm or less dilated. We have therefore divided the material in cases with dilatation of the cervix 2 cm or less, and cases more than 2 cm dilated. When the cervix is more than 2 cm dilated one can easily visualize the membranes and occasionally observe a bulging of the membranes through the cervix. Usually it is not the prolapse of the membranes, but the effacement of the cervix, which makes the operation dif ficult, because it is important for a successful outcome to place the suture as high as possible on the cervix t the level of the internal os. If the membranes are bulg ing the patient is placed in deep Trendelenburg position, and kept well anesthetized. The cervix is then grasped with 2 or 4 sponge sticks, light pulling on the sponge sticks will normally cause the membranes to retract back into the uterine *cavity If not, the membranes* can be pushed up with a sponge stick. Rupture of the membrane during the procedure can be avoided if one keeps a finger inside the cervical os, when the suture is placed.

POSTOPERATIVE TREATMENT

In group 1 where no alcohol was used, the patients were kept in a deep Trendelenburg position for at least 24 hours postoperatively This was followed by bed rest for 3–5 days. The patients were usually given a combination treatment of Sodium Amobarbital (Sodium Amytal) 500 mg every 6 hours and Meperidine (Demerol) 100 mg every 4 hours. The sedation usually continued for 4 days. Some patients would experience mild withdrawal symptoms when the medicine was discontinued. Discharge took place 8–10 days after the operation.

The alcohol group (group 2) was given ethyl alcohol in the dosage described by Fuchs et al. in 1967 an initial *dose of 7.5 ml of 9.5%* alcohol solution per kilogram body weight per hour was given for hours, followed by a maintainance dose of 1.5 ml alcohol solution per kilogram body weight per hour for 6–10 hours. With this treatment a blood alcohol concentration between 0.1? 0.16% was obtained. The intravenous alcohol treatment was started in the Recovery room as soon as the vital signs were stable after the operation. The patients were kept in a mild Trendelenburg position for 12 24 hours, followed by bed rest 1 2 days, they were usually discharged on the 5th or 6th postoperative day

In the earlier cases the patients were treated ith prophylactic antibiotics a combination of penicillin and streptomycin was generally used. In recent years antibiotics were only used when an infection occurred.

RESULTS

In this series of 143 operations, 52 were carried out between the 13th and 20th week of pregnancy and 91 were done between the 20th and 31st week of gestation. Of the 128 premature and full term deliveries, cesarean section was the method of delivery in 69 cases (54 7%) and vaginal delivery was accomplished in 59 in stances. No operative or anesthetic complications occurred in any of the 143 operations.

The 143 patients delivered a total of 119 live infants, including one set of twins, this is a success rate of 82.5%

Of 86 patients who had cervical dilatation of less than 2 cm 73 delivered a liveborn child (84 9%) of the 57 patients with a cervical dilatation of more than 2 cm, 45 delivered live infants (78.9%) Thus, the success rate is higher when the dilatation of the cervix is less than 2 cms, but the difference is less than one would have expected

In the majority of cases with bulging membranes the outcome was good However one of the main problems in case of bulging membranes was postoperative contractions which in 3 cases *in group 1 resulted in premature rupture of* the membranes, after which the suture had to be removed and the patient aborted. One of these pa tients had twins. Another problem was post operative infection which in 5 cases resulted in

Table II. *Post-conceptional Shirodkar procedures. Treated with heavy sedation (Group 1)*

	Cervix <2 cm dilated	Cervix >2 cm dilated
No previous obstetrical history of abortion	5	10
Previous early abortion	5	5
Previous late abortion and premature delivery	50	31
Procedure before 20 weeks	28	8
Procedure after 20 weeks	32	38
No contractions postoperatively	52	30
Contractions postoperatively	8	16
Abortion within 3 days	1	3
Abortion after 3 days	3	4
Premature delivery with neonatal death	4	2
Premature delivery with liveborn	7	8
Full term delivery	45	29
Abortions	4	7
Vaginal delivery	21	19
Caesarean section	35	20
Live infants	52 in 60 86.7%	37 in 46 – 80.4%
Total 89 live infants in 106 cases = 83 7%		

amnionitis The suture had to be cut in all these cases before the infection could be controlled. In one case in group 1 where postoperative contractions could not be controlled with sedation and the suture was not removed, the patient finally aborted through a rupture in the posterior wall of the cervix. Aside from this, there were no major postoperative complications.

In group 1 (Table II) three abortions occurred within 3 days of the operation and seven abortions later There were 6 premature deliveries with neonatal deaths, 15 premature deliveries with liveborn infants and 74 full term deliveries with birth weight above 2 500 g.

In group 2 (Table III) where prophylactic alcohol was given, there was one abortion within 3 days and one abortion later There were two premature deliveries with neonatal deaths, but both infants were reported to have multiple congenital abnormalities. There were four premature deliveries with liveborn infants, and 15 full term deliveries, giving a total of 19 live infants in 23 cases, which is a success rate of 83%.

In 14 cases (group 3) where the patients were treated with heavy sedation, premature labor threatened later and the patients were then given intravenous alcohol (Table IV) In this group, no abortions occurred within 3 days, but one abortion occurred later There were two premature deliveries with neonatal death, two premature deliveries with live births and eight with full term deliveries, or a total of 10 live infants in the 14 cases (71%). Repeated alcohol courses were required in 10 cases.

DISCUSSION

Most authors recommend that the post-conceptional Shirodkar procedure should be done between the 14th and 18th week of gestation. Analysis of our results indicates that 63 6% were carried out after the 20th week, with a success rate of 86.3% however when possible the procedure should preferably be done between the 14th and 16th week of gestation.

The overall success rate in our series is 82 5% Shirodkar (20, 21) published a success rate of 79 4%

McDonald (10) reported in 1957 a success rate

Table III. *Post-conceptional Shirodkar procedures. ETOH started immediately postoperatively (Group 2)*

	Cervix <2 cm dilated	Cervix 2 cm dilated
No previous obstetrical history of abortion	1	1
Previous early abortion	3	0
Previous late abortion and premature delivery	15	3
Procedure before 20 weeks	12	
Procedure after 20 weeks	7	4
No contractions postoperatively	19	2
Contractions postoperatively	0	2
Abortion within 3 days	1	
Abortion after 3 days	1	
Premature delivery with neonatal death	2	
Premature delivery with live births	2	2
Full term delivery	13	2
Abortions	2	0
Vaginal delivery	10	4
Caesarean section	7	0
Live infants	15 in 19 – 80%	4 in 4 – 100%
Total – 19 live infants in 23 cases = 83%		

Table IV *Post-conceptional Shirodkar procedures Alcohol started when labor occurred (Group 3)*

	Cervix <2 cm dilated	Cervix >2 cm dilated
No previous obstetrical history of abortion	0	1
Previous early abortion	0	1
Previous late abortion and premature delivery	7	5
Procedure before 20 weeks	3	1
Procedure after 20 weeks	4	6
No contractions postoperatively	0	0
Contractions postoperatively	7	7
Abortion within 3 days	0	0
Abortion after 3 days	1	1
Premature delivery with neonatal death	0	2
Premature delivery with liveborn	1	1
Full term delivery	5	3
Abortions	1	1
Vaginal delivery	2	3
Cesarean section	4	3
One course of ETOH	1	3
Repeated causes of ETOH	6	4
Live infants	6 in 7 = 86%	4 in 7 = 57%
Total = 10 in 14 = 71%		

of 47% in 70 cases of cervical incompetence treated with a purse-string suture of silk inserted at the level of the internal os. A majority of the cases were performed at the 20–24 week. In 1963 McDonald (11) published an additional 25 cases with a success rate of 80% in this series the operation was done around the fourteenth week of gestation Barter et al. (1) reported in 1958 a success rate of 72 7% on a series of 2. patients In 1963 they published an additional series of 88 cases with an overall fetal salvage rate of 76% (2) Durfee (4) reported 17 live infants in 24 cases (70 8%) and Picot (16) published a 100% success rate in 6 patients.

Mann (14) reported in 1962 from this department a success rate of 83% in 62 patients in whom cervical incompetence was clinically manifest and who had undergone post-conceptional lower isthmic cerclage. Those of Mann's patients that were operated in 1960 or later are included in our material. Benson & Durfee (3) published in 1965 a fetal salvage rate of 82% in 10 patients in whom a transabdominal cervico-uterine cerclage during pregnancy was performed and Lynn (9) reported the same year a success rate of 80% in 5 patients where an open ligature cerclage technique was used. Hoffmeister et al. (7) reported in 1968 a success rate of 63% in 44 patients, where a modified McDonald procedure was performed, and Seppälä & Vara (22) published in 1971 a fetal salvage rate of 83 2%

CONCLUSION

A post-conceptional Shirodkar operation with the technique modified by Barter et al is a safe and successful operation when performed by an experienced physician but it should be reserved for well documented cases of incompetent cervix. The operation is best done between the 14th and 16th week but can be done up to the 30th week of gestation A Shirodkar cerclage is most successfully performed on a cervix that is less than 2 cm dilated but can be done even when the cervix is partially effaced and 4–5 cm dilated We have had 2 successful cases with cervical dilatation of 6 and 8 cm.

The use of alcohol does not appear to improve the overall success rate significantly but the patients seemed to tolerate the alcohol treatment better than the massive sedation with sodium amytal and demerol used in the earlier cases. It is significant that alcohol was able to control the uterine activity in a number of cases, where the sedation had failed. However 10 of the 14 patients required more than one course of alcohol. There were no complications in the cases where alcohol was used, and the patients stay in the Hospital was shortened by 2–3 days, compared to the cases where sedation was used.

ACKNOWLEDGEMENT

We wish to thank Miss Frances A. Macdonald, Chief Statistician of the Department of Obstetrics and Gynecology for her technical assistance.

REFERENCES

1 Barter R. H., Dusbabek J A Riva, H L & Parks J Surgical closure of the incompetent cervix during pregnancy Amer J Obstet Gynec 75 511 1958.
2 Barter R. H Dusbabek J A Tyndal C M, & Erkenbeck, R. V Further experiences with the Shirodkar operation. Amer J Obstet Gynec 85 792, 1963

3. Benson R. C. & Durfee R. B. Transabdominal cervicouterine cerclage during pregnancy for treatment of cervical incompetency. Obstet Gynec 25 145 1965.
4. Durfee, R. B. Surgical treatment of the incompetent cervix during pregnancy. Obstet Gynec *12*, 91, 1958.
5. Fuchs, F. Fuchs, A.-R., Poblete, V. F. J. & Risk, A. Effect of alcohol on threatened premature labor. Amer J Obstet Gynec 99 627 1967.
6. Hellman, L. M. & Pritchard, J. A. Habitual abortion. *I* Williams Obstetrics, 14th ed. Appleton Century Crofts, New York.
7. Hoffmeister, L. J. Schwartz, W. R., Vonstrak, B. F. & Mortens, W. Suture reinforcement of the incompetent cervix. Amer J Obstet Gynec *101* 58, 1968.
8. Lash, A. F. & Lash, S. R. Habitual abortion. Incompetent internal os of the cervix. Amer J Obstet Gynec 59 68, 1950.
9. Lyon, H. D. Open-ligature emplacement for correction of incompetent cervix during pregnancy. Obstet Gynec 26, 377 1965.
10. McDonald, I. A. Suture of the cervix for inevitable miscarriage. J Obstet Gynaec Brit Emp 64 346, 1957.
11. McDonald, I. Incompetent cervix as cause of recurrent abortion. Amer J Obstet Gynaec Brit Comm 70 105 1963.
12. McGowan, G. W. Cervical incompetence in multiple pregnancy. Obstet Gynec 35 599 1970.
13. Mann, E. C. Habitual abortion. A report in two parts on 160 patients. Amer J Obstet Gynec 77 706, 1959.
14. Mann, E. C., Symposium: The incompetent cervix. Bull Sloane Hosp Women 8, 1962.
15. Mann, E. C., McLaren, W. D. & Hayt, D. B. The physiology and clinical significance of the uterine isthmus. Amer J Obstet Gynec *81* 209 1961.
16. Picot, H. Thompson, H. G. & Murphy C. J. J. Surgical treatment of the incompetent cervix in pregnancy. Obstet Gynec *12* 269 1958.
17. Rooney, B. Congenital cervical incompetence in primigravidas. Amer J Obstet Gynec 86 52, 1963.
18. Raphael, S. L. Incompetent internal os of the cervix (views and reviews). Obstet Gynec 28, 438, 1966.
19. Sherman, A. L. Hormonal therapy for control of the incompetent os of pregnancy. Obstet Gynec 28, 198 1966.
20. Shirodkar V. N. A new method of operative treatment of habitual abortion. Antiseptic *52* 299 1955.
21. Shirodkar, V. N. Surgical treatment of habitual abortions. *I* tendances actuelles en gynécologie et obstétrique. Librairie de l'Université, Georg & Cie S. A 1955 s. 45.
22. Seppala, M. & Vara, P. Standardization of the results of Shirodkar's operations. Acta Obstet Gynec Scand 50, 66, 1971.

Submitted for publication Sept. 14 1972

Fritz Fuchs
Department of Obstetrics and Gynecology
The New York Hospital Cornell Medical Center
530 East 71 Street
New York, N.Y. 10021
USA

Acta Obstet Gynec Scand 52: 83–89 1973

CASE REPORT

PREGNANCY COMPLICATED BY EXTREME HYPERLIPAEMIA AND FOAM-CELL ACCUMULATION IN PLACENTA

F. Henning Nielsen, B. Brock Jacobsen and J. Rolschau

From the Department of Gynecology and Obstetrics D (Heads: J. J. Jacobsen, K. Kristoffersen and O. Thomsen Pedersen), the Department of Medicine M (Heads: J. G. Jacobsen and E. Mogensen), and the Institute of Pathology (Heads: H. E. Christensen, C. Jakobsen, N. K. Jensen and J. Kirkegaard), Odense Hospital, Odense, Denmark

Abstract. A case of extreme hyperlipaemia in a 25-year-old primigravida is reported. The presenting symptom was an attack of acute abdominal pain at term. During this attack triglyceride and serum cholesterol levels were found to be increased excessively. The course of labour was uneventful. Histological examination of the placenta revealed accumulation of lipid-containing macrophages in the intervillous space, a finding which has not been described previously. The patient was kept under observation over a period of 5 months postpartum. During this period the serum lipid concentration decreased, whereas a slight type IV hyperlipaemia persisted.

INTRODUCTION

With the exception of lysolecithin, all serum lipid fractions rise in pregnancy (29). The increases in triglyceride and pre-beta-lipoprotein levels are most pronounced, and this applies both to normal individuals (2, 7, 23) and to patients with hypercholesterolaemia (12, 15) and hypertriglyceridaemia (4, 12). Cholesterol, beta-lipoprotein, phospholipids and free fatty acids also increase considerably in normal pregnancy (2, 3, 29).

The levels increase steadily after the first trimester and the highest values are found at term.

Postpartum the concentration of lipids falls rapidly. Hence, prepregnant levels of free fatty acids are reached in four or five days, and of the remaining lipids in from two to three months (3, 23, 30).

The mechanisms underlying the changes in the lipid concentrations provoked by pregnancy are not fully clarified. There might be an interaction of several different factors. Most likely the predominant factors are hormonal changes occurring in pregnancy (estrogens, placental lactogenic hormone, glucocorticoids, growth hormone) although changes in liver function and carbohydrate metabolism as well as gain in weight may also exert an influence (2, 3, 26, 29). The lipoprotein lipase activity and the postheparin esterase activity are reduced during the last part of pregnancy, but after delivery they rapidly become normal and this might be of particular importance in relation to the changes in triglycerides and free fatty acids (6).

As far as triglycerides are concerned, the increase in serum lipid concentration during normal pregnancy is about 300%, and for the remaining lipids somewhat lower (1, 2, 3, 6, 9, 20, 21, 22, 23, 24, 26, 28, 29, 30). Hence these changes are normal consequences of pregnancy and not a hyperlipaemic disorder.

Extreme hyperlipaemia occurring during pregnancy and labour has been observed on a few occasions only. We had the opportunity of following a pregnant woman who had excessive increases in serum lipids, associated with an acute attack of abdominal pain. Placenta histological changes were revealed which have not been described previously (25).

CASE REPORT

The patient was a 25-year-old primigravida. She had not previously been admitted to hospital and had in all essentials enjoyed good health. She was an only child. There was no history of circulatory disorders or diabetes mellitus in her maternal family. The patient's father was unknown. There was no abuse of tobacco, alcohol or drugs.

Nine months before pregnancy she had had an attack,

Table I Lipid changes after delivery

N are the normal values stated as 95 % range (Mean ± S.D.) for females < 29 years old

Days after delivery	0	5	9	12	25	59	102	120	165
Serum triglyceride (mg/100 ml) *N* = 45–93	5 700	1 [illegible]40	1 320	1 196	341	129	406	308	284
Serum glycerol (mmol/l) *N* = 0.06–0.10	1 42	0.10	0.05	0.09	0 09		0 05	0.06	0 07
Serum cholesterol (mg/100 ml) *N* = 121–223	1 370	492	453	384	361	300	234	218	216
Plasma free fatty acids (μmol/l) *N* = 147–625		330		537					
Serum phospholipids (mmol/l) *N* = 2.00–3 45			7.07	3.32				2.60	

lasting for a few days, of slight intermittent epigastric pain and a similar attack during the eighth month of pregnancy. There had not been jaundice, discoloration of faeces and urine or fever on these occasions. She had not been examined further or referred to hospital because of these attacks.

In other respects her pregnancy was normal, and she was checked regularly by her own doctor or in the prenatal clinic, the last time being one week before calculated term. No signs of complicating disorders had been found. Blood pressure was normal, and the urine contained no protein or sugar.

Two days before the calculated term she was admitted as an emergency case to the maternity ward because of persistent pain with intermittent aggravation lasting for 24 hours. The pain was localized to the epigastric region and radiated into the small of the back. During the 24 hours prior to admission normal stools and urine had been passed, there was no vomiting, jaundice or fever. She had been treated with morphine in her home without any effect on the pain.

Examination on admission revealed her general condition to be unaffected, she was afebrile and pulse and blood pressure were normal. Examination of the abdomen showed slight diffuse epigastric tenderness on palpation, but no resistance, abnormal swellings or dullness. Obstetrical examination revealed normal conditions corresponding to pregnancy at term, the uterus was completely relaxed and not tender. Survey X-ray of the abdomen showed normal conditions as far as the pregnancy was concerned, there were no calcification or signs of pneumoperitoneum. When venous blood was drawn for routine examinations, the plasma was found to be milky after centrifugation.

The pain subsided rapidly and spontaneously and had disappeared completely ten hours after admission. Nineteen hours after admission, labour was induced by means of intravenous infusion containing oxytocin and para cortex. Ten hours later, with the aid of Malmström's vacuum-extractor, she gave birth to a boy in the 1st normal vertex presentation. The infant did not present any signs of perinatal asphyxia, dysmaturity or other abnormalities. Birth weight 3 650 g, length 53 cm. The placenta was delivered spontaneously and appeared normal on gross inspection. The extent of bleeding at delivery was normal, although the blood was remarkably pink. The postpartum course was uneventful without fever or abdominal pain.

Examinations during the puerperium did not reveal any signs of cardiovascular disorders, electrocardiogram and chest X rays were normal and there was equal pulsation in the dorsal arteries of the feet. Normal conditions were found on ophthalmoscopy seven days after delivery; there were no demonstrable xanthoma or xanthelasmata.

Eleven days after delivery the patient was discharged to be observed in the out patient clinic at intervals of about one month for the following 5 months. She had no complaints during this period and nothing abnormal was noted. The body weight before pregnancy was about 77 kg, one week before term 83 0 kg, 10 days after delivery 71 5 kg, and five months later 69.8 kg. Height 163 cm. Cholecystography three months postpartum revealed normal conditions.

3 months after delivery, low carbohydrate diet was prescribed. No other treatment of the hyperlipaemia was given.

Laboratory findings

The changes in the lipid levels during the observation period are presented in Table I. All values were determined after the patient had been fasting for 12 hours. The pronounced decreases in serum triglycerides and cholesterol levels are shown in Fig. 1. Lipoprotein electrophoresis was performed for the first time 12 days postpartum and showed a considerable increase in width of

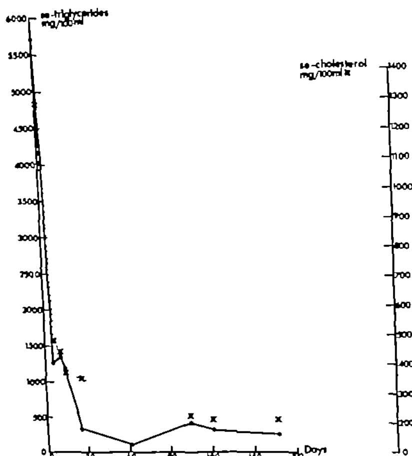


Fig. 1. Lipid changes after delivery

the prebetalipoprotein band. At subsequent examinations this band decreased in width and intensity (Fig. 2).

Other laboratory investigations

On admission and at subsequent examinations the serum amylase was found to be normal. Fasting blood sugar was normal at repeated examinations during her stay in hospital. Intravenous glucose tolerance curve, five days postpartum, was normal (K 1.15). Liver function tests (bilirubin, thymol, prothrombin, alkaline phosphatase, G. O. transaminase, G. P.-transaminase and L. D. H.), as well as thyroid function tests (PBI and T_4) were also normal.

During the entire prenatal and postnatal period, no protein or glucose was found in the urine. During the 24 hour period following admission and during the first postpartum week, there was slight proteinuria (0.05%). On admission there was glucosuria, and the first 24-hour specimen of urine collected contained [illegible] g of glucose per litre, whereas afterwards there was no sugar in the urine. On the day of admission there was pronounced jaundice which disappeared subsequently. During the same period the urinary diastase ranged from 200 to 600 Somogyi units, later it was below 150 units.

Examination of the placenta

Macroscopic examination. The placenta weighed 650 g. It was discoid in shape and of normal proportions. The umbilical cord was inserted centrally on the foetal surface and the vessels branched off normally. Upon cutting the placenta into slices of 1 cm, no grossly visible changes were found. Fixation was done in 4% formalin. After 24 hours the fixation liquid was milky.

Macroscopic examination. Apart from the foam cells described below systematic microscopic examination revealed a normal placenta, and histologically its appearance corresponded to the normal picture at term. However, the vessels in the stem villi were slightly hypertrophic and the endothelium of the arteries was markedly ballooned.

Foam cells

The majority of these cells were lying in the intervillous space in clusters of up to 20 cells, situated at the edge of Langhans, Rohr's and Nitabuch's fibrin layers. At the junction of the two latter layers, the concentration was most pronounced. There were no foam cells in the stroma or vessels of the villi. The foam cells measured about 35 μ, they were polygonal and had an abundant, finely

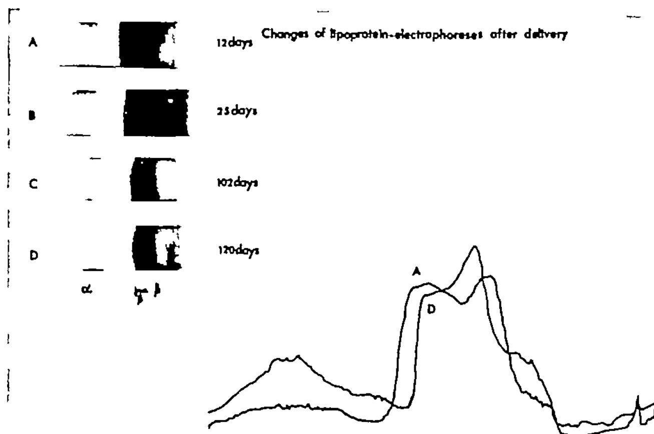


Fig 2

granulated cytoplasm. Most often the nuclei were situated centrally and were rich in chromatin. They stained intensively in the cytoplasm with Sudan Black B and Oil Red. There was faint drop-shaped staining of the adjacent fibrin layers and the syncytial areas (Fig. 3)

The infant

During the neonatal period and at subsequent examinations in the out-patient clinic the infant appeared normal. Unfortunately the infant's serum lipid levels were not determined until four months after birth, and at this time the following normal values were found.

Triglycerides, 177 mg/100 ml, glycerol: 0.05 mM/l

Cholesterol, 136 mg/100 ml, phospholipids, 2.15 mM/l

Serum lipoprotein paper-electrophoresis was also normal.

DISCUSSION

Only very few cases of extreme hyperlipaemia in pregnancy and during labour have been described. The reports available in the literature (4 5 12 14 15 18 27) comprise a total of nine patients. In four of these cases the lipid levels were of the same order of magnitude as those found in our patient and these four cases were complicated by abdominal attacks (5 12 18 27)

In our patient, triglycerides and cholesterol were excessively increased at the time of delivery Similar to the course in normal pregnancies and in the pregnant women with hyperlipaemia mentioned above the lipid concentrations decreased rapidly during the early days postpartum, and subsequently they decreased more slowly The fall in triglycerides was particularly pronounced although these levels did not reach the normal range for non pregnant women during the follow-up period. Cholesterol reached this level about 3 $^1/_2$ months after delivery The postpartum concentrations of free fatty acids and phospholipids correspond to the values described in normal puerperium Twelve days postpartum and during the remaining part of the follow-up period, lipoprotein electrophoresis presented a characteristic type IV pattern (7)

Because lipaemia retinalis or xanthoma was not observed a prolonged or marked increase in the serum lipid levels is not likely to occur The rapid decrease after delivery tends to indicate that it must have been a transitory but pronounced, exacerbation provoked during pregnancy

On admission the patient had an acute ab-



Fig 3 Haematoxylin-eosin, 1 400.

dominal attack with pains in the epigastric region and in the small of the back. Urinary diastase was increased and there were glucosuria and ketonuria However the abdominal attack soon subsided, the patient was afebrile and generally unaffected. There were normal blood sugar and serum amylase levels and only a slight and transitory elev tion of urinary diastase. In several of the cases of extreme hyperlipaemia previously reported similar symptoms were observed (4 5 12, 18 27)

It is well-known that hyperlipaemia (types IV and V) in cases of high lipid concentrations may provok acute abdominal attacks resembling pancreatitis (7 17 19) but it is also known that acute pancreatitis may result in hyperlipaemia of types I IV and V (10, 19) The mild and transitory abdominal attack, taken in conjunction with the very pronounced hyperlipaemia, makes it likely that the hyperlipaemia was the primary factor in our patient No other disorders were found, as e g diabetes mellitus, myxoedema, nephrosis, liver disorders or buse of alcohol, which could have caused a secondary hyperlipaemia.

The slight hyperlipaemia, which persisted five months postpartum, in conjunction with the lipoprotein lectrophoresis pattern, suggests the likelihood that it must have been an essential type IV hyperlipaemia, with exacerbation associated with the influence of pregnancy on the lipid metabolism. It was not possible to prov the existence of hereditary predisposition, because of the familial circumstances mentioned above.

In cases of essential hyperlipaemia, foam cells have been demonstrated in liver spleen, lymph nodes and bone marrow (7 13) but they have not previously been described in the placenta (25) In the above cases of hyperlipaemia occurring in pregnancy the placenta was not examined histologically Foam cells were found only in the maternal part of the placental circulation, which suggests the probability that they occur secondarily to the maternal hyperlipaemia Light microscopy and histochemical examination did not reveal any resemblance between the foam cells and the decidual cells, the syncytiotrophoblasts or the Hofbauer cells Therefore in our opinion the foam cells are free macrophages from the maternal circulation which are accumulated in the inter villous space in the same way as foam cells have been described to accumulate in other organs in cases of hyperlipaemia.

The obliterating endarteritis found cannot further explain the hyperlipaemia, similar changes being found in normal pregnancies and in several maternal disorders (8).

In patients with attacks of acute abdominal pain late in pregnancy hyperlipaemia should be taken into consideration as a possible differential diagnosis. In particular it can be dificult to distinguish between this disorder and acute pancreatitis (11 13 31) which has been proved by the case report presented here. I case reported previously (5), the condition gave rise to exploratory laparatomy and Caesarean section

In cases of severe hyperlipaema, thrombo-embolic

complications are frequently seen. No such complications occurred in our patient, but in a previously reported case of hyperlipaemia in pregnancy deep venous thrombosis and pulmonary embolus developed (18) in another case angina pectoris was observed (12)

Treatment of hyperlipaemia in pregnancy has been discussed only sporadically in the literature In cases of hypercholesterolaemia, low fat diet has been recommended, with addition of unsaturated fatty acids (4 16) as well as plasma-lipid-reducing drugs such as Thyroxin and similar preparations (4 14 16) On the other hand no reports on treatment of hypertriglyceridaemia in pregnancy (particularly types IV and V) are available but a diet low in carbohydrates must be recommended in order to counteract increases in the pre-beta-lipoprotein fractions (7) The diet must be composed so as to meet the requirements of the foetus. In the presence of complications associated with the hyperlipaemia interruption of pregnancy must be taken into consideration and should be recommended, if deemed safe from an obstetrical point of view

After delivery it is recommended to check the hyperlipaemia and if it persists, treatment should be instituted. Hormonal contraceptives should be avoided.

During subsequent pregnancies the patient must be carefully checked because the hyperlipaemia may have a tendency to recur (4 14)

REFERENCES

1 de Alvarez, R. R., Gaiser D F Simkins, D M Smith, E. K. & Bratvold, G E. Serial studies f serum lipids in normal human pregnancy Am J Obst Gynec *77* 743–757 1959

2. Aurell, M. & Cramér K. Serum lipids and lipoproteins in human pregnancy Clin Chim Acta *13* 278–284 1966.

3 Burt, R. L. Plasma nonesterified f tty cids in normal pregnancy and the puerperium. Obstet Gynec *15* 460–464, 1960.

4 Bürger V G Alvarez, O & Reuter W Essentielle Hyperlipämie und Schwangerschaft. Zentralbl Gynak *52* 1766–1770, 1966

5 Drohocki, A. & Rajpert, K. Idiopathic hyperlipaemia as cause of an acute abdominal syndrom in pregnant woman Pol Tyg Lek *24* 1279–1280, 1969

6 Fabian, E., Stork, A Kucerova, L. & Sponarova, J Plasma lev ls f free fatty acids, lipoprotein lipase and postheparin esterace in pregnancy Am J Obst Gynec *100* 904–907 1968.

7 Fredrickson, D S., Levy R. I. & Lees, R. S Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. New Engl J Med *5* 273–281 1967

8. Fox, H. Abnormalities of the foetal stem arteries in the human placenta. J Obstet Gynaec Brit Comm *74* 734–738 1967

9 Green, J G Serum cholesterol changes in pregnancy Am J Obst Gynec *95* 387–393 1966.

10. Greenberger N J Hatch, F T Drummey G. D & Kisselbacher K. J Pancreatitis and hyperlipaemia. A study of serum lipid alterations in 25 patients with acute pancreatitis. Medicine *45* 161 174 1966

11 Jacobs, W A. & Janowitz, H. D., *In* Medical, Surgical and Gynecologic Complications of Pregnancy (ed. J J Rovinsky and A F Guttmacher), pp. 188–189 Livingstone, Edinburgh and London, 1965

12. Kaplan, S., Bossak, E. T Wang, C. & Adlersberg, D Pregnancy complicated by idiopathic hyperlipemia and idiopathic hypercholesterolemia. J Mount Sinai Hosp NY *24* 39–44 1957

13 Kinsell, L. W Schlierf, G Kahlke, W & Schettler G *In* Lipids and Lipidoses (ed. G Schettler), pp. 464–467 Springer Berlin, Heidelberg and New York, 1967

14 Ledvina, M & Skrobal, V Familiäre essentielle Hypercholesterinämie und Schwangerschaft. Zschr Inn Med *21* 156–160, 1966

15 Lee, G B., Culley G. A., Lawson, M. J Adcock, L. L. & Krivit, W Type II hyperlipoproteinemia in mother and twins. Circulation *34* 183–188, 1969

16 Lewis, L. A., Brown, H. B. & Page, I. H. Ten years dietary treatment of primary hyperlipidemia. Geriatrics *16* 64–81 1970

17 Levy R. I & Langer T Mechanisms involved in hyperlipidemia. Modern Treatm 6. 1313–1327 1969

18 Millen, R. S., Russ, E. M Eder H A. & Barr D P Pregnancy complicated by hyperlipaemia. Am J Obst Gynec *71* 326–330, 1956.

19 Moller B. H. Fettinducerad hyperlipämi med letalt förlöpande pancreatitis. Svensk Lakartidn *66* 4158–4160, 1969

20. Nelson, G. H. Serum nonesterified fatty acid levels in human pregnancy as determined by various titration procedures. Am J Obst Gynec *92* 202 206, 1965

21 Olive M F & Boyd, G S Plasma lipid and serum lipoprotein patterns during pregnancy and puerperium. Clin Sci *14* 15–23 1955

22. Peters, J P Heinemann, M & Man, E. B The lipids f serum in pregnancy J Cl Invest *30* 388–394 1951

23 Reboud, P Groulade, J Groslambert, P & Colomb, M. The influence of normal pregnancy and the post partum state on plasma proteins nd lipids Am J Obst Gynec *86* 820–828 1963

24 Robertson, A. F & Sprecher H A review of human placental lipid metabolism and transport. Acta Paed Scand, Suppl. *183* 1–18, 1968

25 Rolschau, J Nielsen, F Hasting & Jacobsen, B Brock Foam cells in the Placenta in xtreme Hyperlipaemia. Acta Path Microbiol Scand (In press).

26 Scandrett, F J Lipid metabolism during pregnancy Mod Trends Hum Reprod P[illegible]

27 Sutter D. E.: Essential hyperlipaemia in pregnancy—a primary cause of acute abdominal pain. Obstet Gynec *17* 587–592, 1961.

28. von Studnitz, W Studies on serum lipids and lipoproteins in pregnancy Scand J Clin Lab Invest *7* 329–333, 1955

29 Svanborg, A. & Vikrot, O Plasma lipid fractions, including individual phospholipids at various stages of pregnancy Acta Med Scand *178* 615–630, 1965

30 Svanborg, A. & Vikrot, O Plasma lipids during the first week after delivery Acta Med Scand *178* 631–636, 1965

31 Walker, B. E. & Diddle, A. W Acute pancreatitis in gynecologic and obstetric practice. Am J Obst Gynec *105* 206–211 1969

Submitted for publication Aug. 1 1972

F Hessing Nielsen
Idrætsallé 9
6733 Hjerting
Denmark

Acta Obstet Gynec Scand 52, 91–92, 1973

NEW INSTRUMENTS

A DEVICE FOR VAGINAL NEEDLE ASPIRATION BIOPSY

Sven Nordvall

From the Department of Obstetrics and Gynecology (Head: Sven Nordvall), Visby General Hospital, Visby, Sweden

Abstract A flexible needle guide for needle aspiration during gynecological examination is described. In 1955 Franzén introduced a special technique for cytological diagnosis of prostatic tumours by transrectal needle aspiration biopsy and he has published several articles about this technique of biopsy from palpable tumours in various organs, such as the thyroid gland, breast, liver salivary gland and other tumours that can be reached with needle.

I 1958 Nils Soderstrom published (4) the results of a large series of aspiration biopsies in more than 800 cases. He has used the method with very wide indications for cytological identification of tumours or other pathological changes in different parts of the human body. The puncture caused no serious discomfort to the patients in his series. He therefore recommended needle aspiration biopsy as safe diagnostic procedure.

There are, howeve two types of possible complications which ought to be taken into consideration. (1) complications due to direct damage by the needle (2) the possibility of spreading the tumour as a result of the puncture.

1 Haemorrhage as complication has not been seen by Soderstrom, nor by me. Puncture of hollow bdominal organs should, according to Söderstrom, be avoided, though probably the fine calibre of the needle prevents serious accidents. No complications of this kind have been observed by Soderstrom nor by me.

2 The risk of disseminating tumour cells is present in all kinds of biopsies but seems very small with a fine needle aspiration. Special care has to be observed during puncture of cystic lesions where leakage of the fluid contents could cause a spread of tumour cells, for example into the abdominal ca ity. When puncturing cyst it is primarily its wall that is of interest, although the fluid often contains cells of diagnostic value

Instrument

A modification of the Franzén needle guide for puncture of the prostate has been developed for needle aspiration biopsy during gynecological ex amination.

The instrument differs from that of Franzén in that the guide is flexible and can follow the movements of the palpating finger. It consists of a short stiff guide for the puncture needle which is fastened to the finger in such a fashion that the distal end of the guide is level with the fingertip. The metal ring that is used for fixation to the finger is split and can thus be adjusted to the size of the finger. The stiff needle-guide is connected

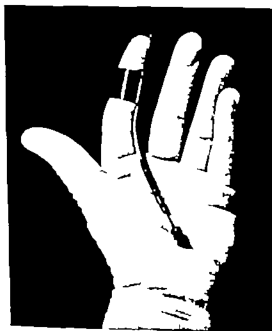


Fig. 1. The device ready for puncture.

to a flexible section along the inside of the hand and index finger It thus allows free movement of the hand and the palpating finger during the ex amination making it possible to find the optimum position for obtaining representative material at the biopsy

Technique

After thorough cleaning of the vagina, a recto-vaginal bimanual palpation is made with the index finger and the needle in the vagina. During palpation the point for the desired aspiration bi opsy is localized and the needle is then advanced into the tissue Suction is applied to the needle which is moved in and out of the tissue. Suction is then released and the needle is withdrawn as rapidly as possible to avoid aspiration of vaginal secretion The cell material in the needle is ejected onto microscope slides, and thin smears are prepared. Fixation is carried out by drying in air or ethanol immersion depending on the type of staining that is to be used

If by aspiration biopsy fluid from a cyst is obtained, the fluid should be mixed with an equal amount of ethanol in order to avoid autolysis. The material obtained by the aspiration biopsy will contain cells from the tissues that have been reached by the needle such as tumour cells, columnar cells, squamous cells mesothelial cells and other forms of epithelium and of course in flammatory cells. In old haemorrhage as for example in endometriosis, macrophages with hae mosiderin will also be present.

The macroscopic appearance of the aspirated material will sometimes allow diagnostic conclusions to be made as for example in cases of extra-uterine pregnancy with intra-abdominal bleeding, in certain inflammatory conditions, in dermoid cysts and in endometriosis.

According to Kjellgren et al (3) the diagnosis obtained after needle aspiration biopsy and cytological examination will correspond to the final pathological diagnosis in 90–95% of cases of ovarian tumour The method will also permit classification of the tumours in many cases.

The device described is manufactured by AB KIFA S-171 95 Solna 1 Sweden.

REFERENCES

1 Franzén, S Giertz, G. & Zajicek, J Cytological diagnosis of prostatic tumours by transrectal aspiration biopsy: A preliminary report. Brit J Urol 3 193 1960.

2. Franzén, S Studier över värdet a punktionscytologi vid diagnostik av tumörsjukdomar Akademisk avhandling. Stockholm 1968.

3 Kjellgren, O., Angstrom, T Bergman, F & Wiklund, D E.. Fine needle aspiration biopsy i diagnosis and classification of ovarian carcinoma. Cancer 8 967 1971

4 Söderström, N Identification of normal tissues and tumours by cytologic aspiration biopsy Acta Soc Med Upsal 63 53 1958.

Submitted for publication February 197

Sven Nordvall
D partment of Obstetrics and Gynecology
Visby Hospital
Visby
S eden

LETTER TO THE EDITOR

Reply to comments of Dr Goodlin, Stanford University School of Medicine
(Acta Obstet Gynec Scand *51* 194)

The comments of Dr Goodlin on our report Changes in Uterine Volume Following the Intra-amniotic Injection of Hypertonic Saline offer an opportunity to clarify points of academic and clinical interest.

Our study strengthened existing evidence (12, 23 24) that after the replacement of 200 ml of amniotic fluid by similar volume of 20% NaCl, there is a roughly 30% increase in the volume of uterine contents. We concluded, on the basis of these and additional data, that this increase in volume promotes the abortifacient action of hypertonic saline. Relying on his own observation, Dr Goodlin considered it appropriate to challenge our findings, to ignore the corroborating evidence of others (12, 23 24) and without specifying his grounds, to criticize our conclusion.

Since the error in our measurements was ~3% and our calibration curve revealed that the instillation of only 100 ml isotonic saline (which did not induce significant uterine activity) increased the area of the amniogram transiently by 1.7 cm² our finding of 30% volume increase, after hypertonic saline treatment, stands valid. A similar method (ultrasonic B-mode sonogram) has been used for the measurement of uterine volume (15) and our results are in good agreement with those of others (12, 23 4). It is, therefore Dr Goodlin's result which stands unsupported and weakened, rather than strengthened, by his personal opinion. It is further weakened by his own statement (13) that: "The uterus changes its shape or contour once contractions begin" for again he overlooked the evidence that soon after the instillation of saline, when the volume increase is already manifest, the increase in cyclic intra-uterine pressure is at yet minimal (18, 25).

The phrase in Dr Goodlin's letter that w added "further confusion to the subject would seem ill-advised, even if it were supported by evidence, rather than only to the "best of [his] knowledge that "within wide limits there is no apparent effect of overdistension of the uterus on initiation of labor" We are not informed by Dr Goodlin how he assembled this "knowledge but it is clear that he ignored the following.

Increase in uterine volume induces through stretch:

(*a*) marked myometrial hypertrophy (19) quantitatively and causally related to stretch (5) and correlated with increased uterine function (11)

(*b*) increase in the mechanic activity of the uterus, as described by the "length-tension relationship (6 21)

(*c*) increase in the generation and propagation of the electric train discharges, which trigger the contractile activity of the myometrium (4 16)

(*d*) increase in the regularity and frequency of the train discharges and thus increase in the rate of rise, magnitude, frequency and regularity of the intra-uterine pressure (10 22)

Dr Goodlin may consider these demonstrations too basic to be useful in his clinical considerations. If so, we would disagree with him. However the evidence documenting the significance of uterine volume (stretch) in the control of uterine activity and thus in the initiation of labor (or abortion) is not limited to the outcome of these basic studies. The effect of litter size on the duration of pregnancy and the initiation of labor was already known to the pioneers (19) and it has been strengthened recently by experiments in which the litter size was controlled (3) The significance of these findings can be dismissed only by the claim that experiments, conducted in animal models, have no bearing whatsoever on clinical considerations. However such an opinion would conflict, with the tradition and structure of modern experimental medicine and

with current emphasis on basic studies in Reproductive Biology

Fortunately the effect of uterine volume on myometrial activity has been examined in patients. Through transabdominal puncture the volume of amniotic fluid has been reduced and increased with isotonic saline (8) Uterine activity decreased and increased respectively as it did in animal experiments (10) The rapid decrease of the intra-uterine pressure following the evacuation of the 1st trimester pregnant uterus (19) or that of the postmenstrual uterus (17) has been prevented by introducing into the uterine cavity by a fluid filled balloon On the removal of this dummy-volume activity decreased (20)

These findings are more difficult to ignore in clinical considerations, for the studies were conducted in patients. However even more directly relevant to the unsupported comments of Dr Goodlin are the well-documented clinical observations that polyhydramnios (1 2, 26) and twin pregnancy (14) precipitate premature delivery and that a mere increase in uterine volume provokes the evolution of uterine activity and abortion in cases of fetal death in utero and missed abortion (7 9)

With this overwhelming evidence at hand, provided by the work of others, it may not be inappropriate to mention a recent observation of our own. We summarize this study here to illustrate the benefits of considering the outcome of basic studies in the design and conduct of clinical trials. We increased the uterine volume of 10 nulliparous midtrimester patients with a high molecular weight dextran solution. We observed that by early rupture of the fetal membranes, 2 patients had treatment failures" but otherwise uterine activity increased in all instances. The stretch-induced evolution of uterine activity reached the desired degree for inducing abortion in 4 patients and caused incipient abortion in 2 leaving 2 women clinically unaffected This method has to be perfected considerably before it might be recommended for routine clinical use. Nevertheless it strengthens the evidence altogether dismissed by Dr Goodlin, that volume increase (stretch) is a *powerful uterine stimulant* not only an implied but also a documented factor in the control of the initiation of abortion and labor

The advice, therefore seems relevant "There are more things in heaven and earth Horatio than are dreamt of in your philosophy"

M O Pulkkinen M D Associate Professor
A Kivikoski M D Assistant Professor
Turku University Medical School
SF-20520 Turku
Finland

REFERENCES

1 Barry A. P Obstet Gynec *11* 667 1958.
2 Buckingham, J C., McElin, T W Bowers, V M. & McVay J Obstet Gynec *15* 652, 1960.
3 Csapo, A. I *In* Ciba Foundation Study Group No. 34 Progesterone its Regulatory Effect on the Myometrium, 1969
4 Csapo, A. I. The electrophysiological manifestations of the progesterone effect on the uterus, Chapter 5 *In* The International Encyclopedia of Pharmacology and Therapeutics (ed. M Tausk), Pergamon Press, New York, 1971
5 Csapo, A. I Erdos, T De Mattos, C. E. R., Gramss, E. & Moscowitz, C. Nature 207 1378, 1965
6. Csapo, A. I. & Goodall, M. C. J Physiol *126* 384, 1954
7 Csapo, A. I Jaffin, H., Kerenyi, T De Mattos, C. E. R. & Souza-Filho, M. B. Amer J Obstet Gynec *87* 892, 1963
8. Csapo, A. I., Jaffin, H., Kerenyi, T Lipman, J & Wood, C. Amer J Obstet Gynec *85* 819 1963.
9 Csapo A. I., Sauvage, J P & West, W G. Amer J Obstet Gynec *108* 6, 950 1970.
10. Csapo, A. I., Takeda, H. & Wood, C. Amer J Obstet Gynec *85* 813 1963.
11 De Mattos, C. E. R., Kempson, R. L., Erdos, T & Csapo, A. I. Fertil Steril *18* 545 1967
12. Gochberg, H. S. & Reid, D E Obstet Gynec *27* 648 1966
13 Goodlin, R. C Kresch, A. J & Northway W H Obstet Gynec *31* 40, 1968
14. Guttmacher A. F & Kohl, S. L. Obstet Gynec *1* 528, 1958.
15 Hellman, L. M Kobayashi, M Fillisti, L. & Lavenhar M. Amer J Obstet Gynec *103* 789 1969
16 Kuriyama, H *In* Ciba Foundation Study Group No. 9 Progesterone and the Defense Mechanism of Pregnancy 1961
17 Macedo Costa, L. Professional Thesis published by the University of Bahia, Brazil
18. Pulkkinen, M O & Kivikoski, A. Int J Obstet Gynec *7* 279 1969
19 Reynolds, S. R. M Physiology of the Uterus, Second Edition. Hoeber Inc., New York, 1949
20 Rottner B *In* Ciba Foundation Study Group No. 34, Progesterone, its Regulatory Effect on the Myometrium, 1969

21. Schofield, B. M. & Wood, C.: J Physiol *175* 125, 1964.
22. Takeda, H.: Fertil Steril *16* 113, 1965.
23. Wagner, G.: Clin Obstet Gynec 9: 520, 1966.
24. Wagner, G.: Acta Obstet Gynec Scand 50, Suppl. 9: 44, 1971.
25. Wiqvist, N. & Eriksson, L.: Amer J Obstet Gynec *88* 35, 1964.
26. Yordon, E. & D'Esopo, D. A.: Amer J Obstet Gynec 70: 266, 1965.

ANNOUNCEMENTS

IAEA Symposium

Ths IAEA Symposium: *Radioimmunoassay and Related Proced res in Clinical Medicine and Research* will be held in Istanbul, Turkey 4-8 June 1973

O ganizers. International Atomic Energy Agency Kärntner Ring 11-13 A 1010 Vienna, Austria.

Scientific Secretaries. Dr E. J Garcia and Dr E. H Belcher Medical Applications Section.

The programme of this Symposium, the second to be organized by the International Atomic Energy Agency in the subject field, will cover all aspects of radioimmunoassay and related procedures and their applications. Particular attention will be given to the in vitro deter mination of substances of clinical interest, such as hor mones, vitamins and drugs. Sections of the programme will be devoted to recently developed techniques for the detection of tumour viral and bacteral antigens, and to essays employing labelled antibodies and naturally oc curring specific receptors.

Further information will be obtainable from national authorities for atomic matters. Abstracts of papers, intended for presentation at the Symposium must be submitted to the International Atomic Energy Agency through these authorities.

IX Acta Endocrinologica Congress

Th IXth Acta Endocrinologia Congress will be held in Oslo June 17 1 1973

President Dr Jørgen H. Vogt, Medical Department B, Aker Hospital, Oslo 5

Secretary General. Dr Asbjørn Aakvaag, Hormone & Isotope Laboratory Aker Hospital, Oslo 5

Enquiries should be addressed to the Secretary General.

VII World Congress of Obstetrics and Gynaecology

The VIIth World Congress of Obstetrics and Gynaecology takes place in Moscow USSR, on August 1 18 1973.

The Congress agenda includes the following topics problems of obstetrics and gynaecology

1 Physiology and pathology of the contractile function of the uterus.

– Biochemistry of amniotic fluid, the fetus and new born.

3 The influence of hormones on tumor growth and development in women.

4 Childhood gynaecology

5 The latest achievements in obstetrics and gynaecology

Address of the Secretariat of the National Organizing Committee of the VIIth World Congress of Obstetrics and Gynaecology All-Union Scientific Research Institute of Obstetrics and Gynaecology – Yelanski St., Moscow G-435 119873 USSR.

The Reinier de Graaf Tercentenary Symposium

The Reinier de Graaf Tercentenary Symposium ill be held August 9 10 and 11 1973 in Delft, the Netherlands, organized by the Society of Obstetrics and Gynaecology of the Netherlands. (Secretary Professor Dr T K. A B Eskes, St Radboud Hospital, Nijmegen.)

Lectures will be presented on several aspects of ovarian function. A limited number of free communications can be accepted (before April 1st, 1973).

BOOKS RECEIVED

Radioterapia Ginecologica by Mario Lenzi and Renato Bergonzini. Edizioni Minerva Medica, Modena 1972. 355 p. Price 12 000 lire.

From the Radiological Institute the University of Modena. Very well illustrated.

Illustrated Human Embryology vol 1 Embryogenesis by H. Tuchmann-Duplessis, G David and P Haegel. Springer Verlag, New York, 1972. 110 p.

An extremely well illustrated booklet, which can be highly recommended to medical students.

International Encyclopedia of Pharmacology and Therapeutics edited by M Tausk Section 48 volumes I and II Volume I published Sept. 1971 517 p. Price £10 00 Volume II published Sept. 1972, 538 p. Price £12.00. Pergamon Press, Ltd, Oxford.

The two volumes give a most valuable and actual survey concerning the pharmacology of the endocrine system and related drugs. They deal with progesterone progestational drugs and antifertility agents. Professor Tausk, who has cted as the edito gives an excellent historical introduction and is also the author of several chapters concerning progesterone in cooperation with doc tor Visser Other authors, well-known in Scandinavia, are Docto Rudel and Doctor Kincl, Professor Ferin and Professor Semm.

Biology I Mammalian Fertilization and Implantation, edited by Kamran S. Moghissi and E. S. E. Hafez. Charles C. Thomas, Springfield, Illinois, 1972. 509 p.

The book is an up-to-date compendium of the nov lies in reproductive biology and present the Proceedings of the VIth Harold C Mack Symposium, held in Detroit October 29–30, 1970. The volume deals with capacitation, fertilization and implantation, and has the same high tandard as the previous ones

√ *The Use of Non-human Primates in Research on Human R production,* edited by E. Diczfalusy and C. C. Standley 536 p.

The book is published as supplementum No. 166 of Acta Endocrinologica Periodica, Copenhagen, 1972, but can lso be obtained from the WHO Research and Training Centre on H ma Reproduction, Karolinska sjukhuset, S-104 01 Stockholm 60, S eden.

It contains the Proceedings f Symposium on the subject i Sukhumi, USSR, i December 1971

Acta Obstet Gynec Scand 52: 97–101 1973

FERTILITY OF DONORS AT HETEROLOGOUS INSEMINATION

Magnar Ulstein

From the Department of Obstetrics and Gynecology, Östra Sjukhuset (Head, Professor Per Bergman), Göteborg, Sweden

Abstract. Seminal properties in relation to fertility were studied in 14 semen donors, used for heterologous inseminations in 178 women. There were 100 conceptions. Fertility of the donors was statistically significantly related to the in vitro penetration and duration of motility of spermatozoa in cervical mucus.

The ethical, legal, and religious aspects of heterologous insemination have been much discussed. Even today there is much adverse criticism of this treatment, but in several countries heterologous insemination has become accepted as a rational method for circumventing sterility in certain barren marriages. The indications are found in cases of permanent male infertility with an apparently fertile female, in marriages where the husband is a carrier of hereditary disease, and cases where the wife is Rhesus negative with a history of stillborn hydropic infants, and the husband is homozygous Rhesus positive. For the last-mentioned cases insemination by a Rhesus negative donor is indicated.

For heterologous insemination fertile donors with high seminal quality are required. Evidence from this and other works (3, 4, 5) shows that there are some differences with regard to the fertility of the donors. The routine seminal analysis usually gives no explanation of the differences.

The aim of this investigation was to make a detailed study of donor semen quality including in vitro penetration and duration of motility of spermatozoa in cervical mucus, and to relate the results to the rate of conception as an index of fertility of the donors.

MATERIAL

In this series 14 donors were used. They were all married and had fertile children. They were physically and mentally healthy without hereditary diseases in the family. The semen quality was normal for all of them. Blood grouping was undertaken, body build, hair color, and color of the eyes were noted and taken into consideration when it came to choice of donor for the different patients.

The 178 women for whom the inseminations were performed were married to infertile men. Careful investigation of the men was undertaken and the indications for insemination are shown in Table I. For women with normal gynecological findings on clinical examination detailed investigation was performed only if the first 3 inseminations failed. Women with pathological findings which might result in decreased fertility are not included in this series. Ages of the women at start of the treatment ranged from 23 to 35 years.

METHODS

Seminal investigations. Routine semen analysis was regularly performed for the donors. The semen samples were taken by masturbation and brought to the hospital in plastic condoms, specially made for this use. The analyses included specification of volume, density, motility, morphology, content of fructose and acid phosphatases in seminal plasma. For a more detailed description see previous paper (10). At each insemination volume, density and motility were specified.

Penetration test. The in vitro penetration test was performed by the method of Kremer (8) with slight modifications (10). Cervical mucus of ovulatory character, fulfilling certain criteria was used as test medium (10). The cervical mucus was drawn into small capillary tubes and one end immersed into the semen reservoir. Penetration time was 3 hours and incubation temperature 37°C. The penetration extent of the foremost spermatozoon was given in mm and taken as a measure for penetration.

Duration of motility. The duration of motility of spermatozoa in cervical mucus was tested in the same tubes as those used for penetration tests. After reading the penetration, incubation at 37°C was continued and at intervals examination of the motility was performed. The duration of sperm motility was classified as follows: < 48 hours, 48–72 hours, and > 72 hours. For each donor the mean values of several semen analyses, penetration tests, and tests for duration of motility are given in the tables.

ANNOUNCEMENTS

IAEA Symposium

The IAEA Symposium *Radioimmunoassay and Related Procedures in Clinical Medicine and Research* will be held in Istanbul, Turkey 4–8 June 1973

Organizers. International Atomic Energy Agency Karntner Ring 11–13 A 1010 Vienna, Austria.

Scientific Secretaries. Dr E. J Garcia and Dr E. H. Belcher Medical Applications Section.

The programme of this Symposium, the second to be organized by the International Atomic Energy Agency in the subject field, will cover all aspects of radioimmunoassay and related procedures and their applications. Particular attention will be given to the in vitro determination of substances of clinical interest, such as hormones, vitamins and drugs. Sections of the programme will be devoted to recently developed techniques for the detection of tumour viral and bacterial antigens, and to assays employing labelled antibodies and naturally occurring specific receptors.

F rther information will be obtainable from national authorities for atomic matters. Abstracts of papers, intended for presentation at the Symposium must be submitted to the International Atomic Energy Agency through these authorities.

IX Acta Endocrinologica Congress

The IXth Acta Endocrinologia Congress will be held in Oslo June 17 1 1973

President Dr Jørgen H. Vogt, Medical Department B, Aker Hospital, Oslo 5

Secretary General. Dr Asbjørn Aakvaag, Hormone & Isotope Laboratory Aker Hospital, Oslo 5

Enquiries should be addressed to the Secretary General

VII World Congress of Obstetrics and Gynaecology

The VIIth World Congress of Obstetrics and Gynaecology takes place in Moscow USSR, on August 1 11, 1973.

The Congress agenda includes the following urgent problems of obstetrics and gynaecology

1 Physiology and pathology of the contractile function of the uterus.

2 Biochemistry of amniotic fluid, the fetus and newborn.

3 The influence of hormones on tumor growth and development in women.

4 Childhood gynaecology

5 The latest achievements in obstetrics and gynaecology

Address of the Secretariat of the National Organizing Committee of the VIIth World Congress of Obstetrics and Gynaecology All-Union Scientific Research Institute of Obstetrics and Gynaecology 2, Yelanski St., Moscow G-435 119873, USSR.

The Reinier de Graaf Tercentenary Symposium

The Reinier de Graaf Tercentenary Symposium will be held August 9 10 and 11 1973, in Delft, the Netherlands, organized by the Society of Obstetrics and Gynaecology of the Netherlands. (Secretary Professor Dr T K. A. B. Eskes, St Radboud Hospital, Nijmegen.)

Lectures will be presented on several aspects of ovarian function. A limited number of free communications can be accepted (before April 1st, 1973).

BOOKS RECEIVED

Radioterapia Ginecologica by Mario Lenzi nd Renato Bergonzini. Edizioni Minerva Medica, Modena 1972. 355 p. Price 12 000 lire.

From the Radiological Institute the University of Modena. Very well illustrated.

Illustrated Human Embryology vol. 1 Embrygenesis by H. Tuchmann-Duplessis, G David nd P Haegel. Springer Verlag, New York 1972. 110 p

An extremely well illustrated booklet, which can be highly recommended to medical students.

I ternational Encyclopedia of Pharmacology and Therapeutics, edited by M T usk. Section 48 volumes I and II Volume I, published Sept. 1971 517 p. Price £10.00. Volume II, published Sept. 1972, 538 p. Price £1 00 Pergamon Press, Ltd, Oxford.

The two volumes give a most valuable and actual survey concerning the pharmacology of the endocrine system and related drugs. They deal with progesterone, progestational drugs and antifertility gents. Professor Tausk, who has acted as the editor gives an excellent historical introduction and is also the author of several chapters concerning progesterone in cooperation with doctor Vreser Othe authors, well-known in Scandinavia, are Doctor Rudel nd Doctor Kincl, Professor Ferin and Professor Semm.

Biology of Mammalian Fertilization and Implantation, edited by Kamran S. Moghissi and E. S. E. Hafez. Charles C. Thomas, Springfield, Illinois, 1972. 509 p

The book is an up-to-date compendium of the novelties in reproductive biology and presents the Proceedings of the VIth Harold C Mack Symposium, held in Detroit October 29–30, 1970 The volume deals with capacitation, fertilization and implantation, and has the same high standard as the previous ones

Th Use of Non-human Primates in R search on Human R production, edited by E. Diczfalusy and C. C. Standley. 536 p.

The book is published as supplementum No. 166 of Acta Endocrinologica Periodica, Copenhagen, 1972, but can also be obtained from the WHO Research and Training Centre on Human Reproduction, Karolinska sjukhuset, S-104 01 Stockholm 60 S eden.

It contains the Proceedings f a Symposium on the subject in Sukhumi, USSR, in December 1971

Acta Obstet Gynec Scand 52 97–101 1973

FERTILITY OF DONORS AT HETEROLOGOUS INSEMINATION

Magnar Ulstein

From the Department of Obstetrics and Gynecology Östra Sjukhuset (Head Professor Per Bergman), Göteborg, Sweden

Abstract Seminal properties in relation to fertility were studied in 14 semen donors, used for heterologous inseminations in 178 cases. There were 100 conceptions. Fertility of the donors was statistically significantly related to the in vitro penetration and duration of motility of spermatozoa in cervical mucus.

The ethical, legal, and religious aspects of heterologous insemination have been much discussed. Even today there is much adverse criticism of this treatment, but in several countries heterologous insemination has become accepted as a rational method for circumventing sterility in certain barren marriages. The indications are found in cases of permanent male infertility with an apparently fertile female, in marriages where the husband is carrier of hereditary disease, and cases where the wife is Rhesus negative with a history of stillborn hydropic infants, and the husband is homozygous Rhesus positive. For the lastmentioned cases insemination by a Rhesus negativ donor is indicated

For heterologous insemination fertile donors with high seminal quality are required. Evidence from this and other works (3 4 5) shows that there are some differences with regard to the fertility of the donors The routine seminal analysis usually gives no explanation of the differences

The aim of this investigation was to make a detailed study of donor semen quality including in vitro penetration and duration of motility of spermatozoa in cervical mucus, and to relate the result to the rate of conception as an index of fertility of the donors

MATERIAL

In this series 14 donors ere used. They were all married and had red children They were physically and mentally healthy without hereditary diseases in the family The semen quality was normal for all of them. Blood grouping was undertaken, body build, hair color and color of the eyes ere noted and taken into consideration when it came to choice of donor for the different patients.

The 178 women for hom the inseminations ere performed ere married to infertile men. Careful investigation of the men was undertaken and the indications for insemination are shown in Table 1. For women with normal gynecological findings on clinical examination detailed investigation was performed only if the first 3 inseminations failed Women with pathological findings which might result in decreased fertility are not included in this series Ages of the women at start f the treatment ranged from 23 to 35 years.

METHODS

Seminal investigations. Routine semen analysis was regularly performed for the donors. The semen samples were taken by masturbation and brought to the hospital in plastic condom, specially made for this use The analysis included specification of. volume, density motility morphology content of fructose and acid phosphatases in seminal plasma For more detailed description see previous paper (10). At each insemination volume, density and motility were specified.

Penetration test The in vitro penetration test was performed by the method of Kremer (8) ith slight modifications (10). Cervical mucus of ovulatory character fulfilling certain criteria as used as test medium (10). The cervical mucus was draw into small capillary tubes and one end immersed into the semen reservoir. Penetration time was 3 hours and incubation temperature 37°C. The penetration extent of the foremost spermatozoa was given in mm and taken as measure for penetration

Duration f motility The duration of motility of spermatozoa in cervical mucus as tested in the same tubes as those used for penetration tests. After reading the penetration, incubation at 37°C was continued and at intervals examination of the motility was performed. The duration of sperm motility was classified as follow 48 hours, 48–72 hours, and 72 hours. For each donor the mean values of several semen analyses, penetration tests, and tests for duration of motility are given in the tables.

Table I. *Reasons for heterologous insemination*

	Number
Azoospermia	112
Oligospermia	19
Oligo-asthenospermia	43
Autoimmunization to spermatozoa	4

Insemination. Fresh ejaculates were used, never later than 1 hour after ejaculation. Ovulation time was estimated by temperature charting over several cycles, the length of cycle and examination of the cervix and the cervical mucus. The insemination was by intracervical injection combined with cap application. For the cervical application a plastic tubing was inserted into the lower part of the cervical canal and 0.5 ml of the ejaculate was injected. The rest of the ejaculate was deposited into a cervical cap covering the portio (11). The plastic cap was furnished with a tube through which the semen could be injected. On 23 occasions an ejaculate was divided for use in 2 patients. Never more than 1 insemination per cycle was performed. The patients rested in the lithotomy position for 1 hour after the insemination, and the plastic cap was removed after 1–8 hours.

Statistical calculations For studying the relation between semen properties and conception rate correlation coefficients were calculated and significance established at the 5% level.

RESULTS

Of the 178 patients inseminated 88 conceived, 77 conceived once 10 twice and 1 patient 3 times, making a total of 100 pregnancies. Pertinent data are given in Table II

Fig. 1 shows the results in relation to the number of inseminations and the cumulative frequency of conceptions. Twenty-eight conceptions occurred with the first insemination 56 within 3 cycles, and 84 within 6 cycles of treatment. An average of 3 6 inseminations was required to attain conception

Table II *Data on 810 heterologous inseminations performed at Östra sjukhuset Göteborg*

	Number
Inseminations	810
Patients treated	178
Conceptions	100
Patients with 1 conception	77
Patients with 2 conceptions	10
Patients with 3 conceptions	1
Births including 1 set of twins	61
Live, healthy babies	62
Spontaneous abortions	8
Pregnant at time of report	31

Table III shows the relation between cycle length and the day of insemination in cycles when conception occurred. For women with 28-day cycles most of the conceptions occurred following insemination on the 13th day of the cycle.

Table IV shows the number of conceptions, number of inseminations, and the conception index for the different donors. The conception index was calculated according to the following equation

$$\text{conception index} = \frac{\text{number of conceptions} \times 100}{\text{number of inseminations}}$$

Table V shows the semen properties of the donors expressed as mean values.

Correlations between semen properties and conception index are given in Table VI The correlation coefficients for most of the semen properties were very low Clearly the highest correlation was found for penetration in cervical mucus, and the next highest was the correlation between conception index and duration of sperm motility in cervical mucus. Testing of significance showed that only the *r* values for sperm penetration and duration of sperm motility in cervical mucus were *statistically different from 0* Sperm penetration showed a statistically significantly higher correlation to conception index than the duration of sperm motility in cervical mucus.

DISCUSSION

Reports on heterologous insemination usually deal with small series, but a few larger series with several hundred patients have also been reported (2, 6) The frequency of conceptions has universally been high (2, 3 4 5 6, 7) Behrman (1) in a survey of earlier work found that 61% patients conceived. Comparison of results from different series is difficult. The fertility of the donors varies, and the criteria for selection of patients differs. The technic of treatment estimation of ovulation time and number of inseminations per cycle vary In the present series the frequency of conception is about the same as that of other series.

Potter (9) stated that the number of inseminations per cycle is of greater importance than the technic. Behrman (3) found no reason for doing inseminations only once a month except for experimental purposes. In the present series only

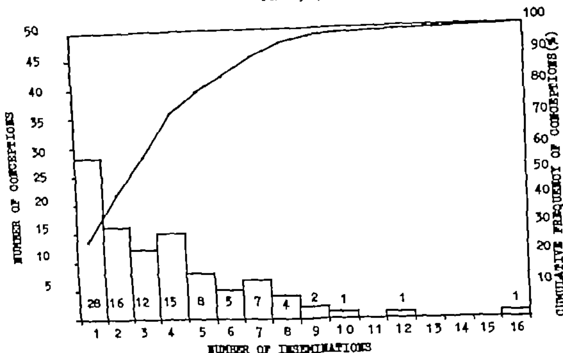


Fig. 1. Number of cumulative frequency of conceptions plotted against number of inseminations.

one insemination was performed per cycle in apparently fertile women and the results are similar to those in series where several inseminations were performed during each cycle.

Determination of ovulation time is difficult, and better results could possibly be obtained if hormone analysis, vaginal smears and other methods were included. However the high percentage of conceptions in the first cycle of insemination in this series indicates that for practical purposes, estimation of ovulation time by means of basal body temperature chart, length of cycle, cervical findings, and character of the cervical mucus is quite satisfactory.

The high incidence of conceptions within the first 3 months of treatment indicates that a trial period over 3 months should be accepted before complete infertility investigations are performed on the female partner.

From the present investigation it seems likely that the donors differed in fertility degree. If all inseminations were performed about the time of ovulation, this difference in fertility could be exactly stated. Several factors of uncertainty are

Table III. *Day of conception for different cycle lengths*

Length of cycle (days)	Day of cycle 10	11	12	13	14	15	16	17	18
24–27	3	7	6	1					
28		15	23	4	1				
29–32				7	10	9	4	2	
33–35						2	4	1	1

Table IV. *Number of inseminations, number of conceptions, and conception index of the donors*

Donor	Inseminations (no.)	Conceptions (no.)	Conception index
A	27	4	14.8
B	86	6	6.9
C	102	3	2.9
D	142	13	9.2
E	11	1	9.0
F	103	13	12.6
G	67	6	8.9
H	83	19	22.9
I	34	5	14.7
J	14	1	7.1
K	48	12	25.0
L	41	5	12.2
M	23	6	26.1
N	29	6	20.9
Total	810	100	

Table I *Reasons for heterologous insemination*

	Number
Azoospermia	112
Oligospermia	19
Oligo-asthenospermia	43
Autoimmunization to spermatozoa	4

Insemination Fresh ejaculates were used, never later than 1 hour after ejaculation. Ovulation time was estimated by temperature charting over several cycles, the length of cycle and examination of the cervix and the cervical mucus. The insemination was by intracervical injection combined with cap application. For the cervical application a plastic tubing was inserted into the lower part of the cervical canal and 0.5 ml of the ejaculate was injected. The rest of the ejaculate was deposited into a cervical cap covering the portio (11). The plastic cap was furnished with a tube through which the semen could be injected. On 23 occasions an ejaculate was divided for use in 2 patients. Never more than 1 insemination per cycle was performed. The patients rested in the lithotomy position for 1 hour after the insemination, and the plastic cap was removed after 1-8 hours.

Statistical calculations For studying the relation between semen properties and conception rate, correlation coefficients were calculated and significance established at the 5% level.

RESULTS

Of the 178 patients inseminated 88 conceived, 77 conceived once, 10 twice and 1 patient 3 times, making a total of 100 pregnancies. Pertinent data are given in Table II

Fig. 1 shows the results in relation to the number of inseminations and the cumulative frequency of conceptions. Twenty-eight conceptions occurred with the first insemination 56 within 3 cycles, and 84 within 6 cycles of treatment. An average of 3 6 inseminations was required to attain conception.

Table II *Data on 810 heterologous inseminations performed at Östra sjukhuset Göteborg*

	Number
Inseminations	810
Patients treated	178
Conceptions	100
Patients with 1 conception	77
Patients with 2 conceptions	10
Patients with 3 conceptions	1
Births including 1 set of twins	61
Live, healthy babies	62
Spontaneous abortions	8
Pregnant at time of report	31

Table III shows the relation between cycle length and the day of insemination in cycles when conception occurred For women with 28-day cycles most of the conceptions occurred following insemination on the 13th day of the cycle.

Table IV shows the number of conceptions, number of inseminations, and the conception index for the different donors. The conception index was calculated according to the following equation

$$\text{conception index} = \frac{\text{number of conceptions} \times 100}{\text{number of inseminations}}$$

Table V shows the semen properties of the donors expressed as mean values.

Correlations between semen properties and conception index are given in Table VI The correlation coefficients for most of the semen properties were very low Clearly the highest correlation was found for penetration in cervical mucus, and the next highest was the correlation between conception index and duration of sperm motility in cervical mucus. Testing of significance showed that only the *r*-values for sperm penetration and duration of sperm motility in cervical mucus were statistically different from 0 Sperm penetration showed a statistically significantly higher correlation to conception index than the duration of sperm motility in cervical mucus.

DISCUSSION

Reports on heterologous insemination usually deal with small series, but a few larger series with several hundred patients have also been reported (2 6) The frequency of conceptions has universally been high (2, 3 4 5 6 7) Behrman (1) in a survey of earlier work found that 61% patients conceived. Comparison of results from different series is difficult. The fertility of the donors varies, and the criteria for selection of patients differs. The technic of treatment estimation of ovulation time and number of inseminations per cycle vary In the present series the frequency of conception is about the same as that of other series.

Potter (9) stated that the number of inseminations per cycle is of greater importance than the technic. Behrman (3) found no reason for doing inseminations only once a month except for experimental purposes In the present series only

3. — Techniques of artificial insemination. Progress in Infertility (ed. S. J. Behrman & R. W. Kistner). Little, Brown & Co., Boston, 1968.
4. Cary W. H.: Results of artificial insemination with an extramarital specimen. Amer J Obstet Gynec *56*, 727 1948.
5. Guttmacher A. F. Role of artificial insemination in treatment of sterility. Obstet Gynec Survey *15*: 767 1960
6. Haman, J. O.. Therapeutic donor insemination. Review of 440 cases. Calif Med *90*: 130, 1959
7. Kleegman, S. J. Therapeutic donor insemination. Fertil Steril *5* 7 1954.
8. Kremer, J. A simple sperm penetration test. Int J Fertil *10*: 209, 1965.
9. Potter R. G., Artificial insemination by donors. Analysis of seven series. Fertil Steril *9* 37 1958.
10. Ulstein, M.. Sperm penetration of cervical mucus as criterion of male fertility. Acta Obstet Gynec Scand *51* 335, 1972.
11. Whitelaw M. J. Use of the cervical cap to increase fertility in cases of oligospermia. Fertil Steril *1*: 33 1950.

Submitted for publication Febr. 22 1972

Magnar Ulstein
Fylkessjukehuset i Ålesund
6017 Åsestranda
Norway

Table V *Semen properties of the donors expressed as means values*

Donor	Volume (ml)	Density (mill. per ml)	Living (%)	Motile (%)	Motility degree	Abnormal (%)	Fructose (mg %)	Acid phosphatase (thousands IE/ml)	Penetration (mm/3 h)	Duration of motility (h)
A	2.8	92	81	55	3.2	36	375	71	34	<48
B	6.2	71	77	57	3.4	43	415	43	19	<48
C	3.1	72	72	52	3.1	45	265	21	16	<48
D	2.8	107	76	57	3.0	37	283	34	18	48–72
E	3.8	162	81	61	3.5	42	321	45	17	<48
F	3.1	206	82	62	3.3	38	524	25	29	48–72
G	4.6	115	79	55	3.9	32	311	51	35	<48
H	5.3	146	77	62	3.1	37	165	24	40	>72
I	3.2	125	87	56	3.0	32	410	56	25	48–72
J	3.4	143	81	52	3.3	30	135	23	24	>72
K	2.6	181	76	59	3.2	31	367	59	40	>72
L	4.6	81	87	54	3.5	33	281	72	19	48–72
M	4.1	84	79	57	3.4	46	221	51	40	>72
N	5.7	91	72	51	3.2	42	325	25	40	<48

connected with this question. If in retrospect all inseminations, performed 3 days prior to and on the day of the temperature rise were selected, the conception index should be more reliable. Such a selection was made and it was found that the conception indices for the donors were then a little higher. Relative differences in conception index between the donors remained unchanged and the correlation between sperm penetration and the conception index was about the same as that found in the series overall. Inseminations performed within these limits or outside them were equally distributed for all donors.

From this it may be concluded that the donors had an equal opportunity to produce conception and the conception index may be accepted as an indicator of fertility.

Table VI *Correlation between semen properties and conception index*

Semen property	Correlation coefficient
Volume of ejaculate	0.18
Density of spermatozoa	0.24
Percentage of living spermatozoa	0.26
Percentage of motile spermatozoa	0.46
Motility degree	0.16
Percentage of abnormal spermatozoa	−0.34
Content of fructose in seminal plasma	−0.08
Content of acid phosphatase in seminal plasma	0.16
Sperm penetration in cervical mucus	0.84
Duration of sperm motility in cervical mucus	0.55

The donors had semen quality within the limits of normality and all of them were fertile. An explanation of the varying fertility was first sought in the usual semen analysis. The highest correlation was found between percentage of motile spermatozoa and conception index, but this was not significantly different from 0. The tests of in vitro sperm penetration and duration of sperm motility in cervical mucus which are included in the present study have not earlier been performed in connection with heterologous insemination. Both investigations showed higher correlations to the conception index than the values for the other semen properties, and the correlation coefficients were significantly different from 0. For sperm penetration the correlation coefficient was significantly higher than for duration of sperm motility in cervical mucus.

In a previous paper (10) it was shown that sperm penetration has a higher discriminating power for fertility or infertility than the other semen properties and only 43 per cent of the variance of penetration was due to regression of the other semen properties. The present results also indicate that the sperm penetration in cervical mucus gives information on fertility not obtained from routine semen analysis, even in a group of fertile men.

REFERENCES

1 Behrman, S. J. Artificial insemination. Fertil Steril *10*, 48, 1959.
— Artificial insemination. Int J Fertil 6, 291, 1961.

3. — Techniques of artificial insemination. Progress in infertility (ed. S. J. Behrman & R. W. Kistner). Little, Brown & Co., Boston, 1968.
4. Cary W. H.: Results of artificial insemination with an extramarital specimen. Amer J Obstet Gynec 56: 727, 1948.
5. Guttmacher A. F.: Role of artificial insemination in treatment of sterility. Obstet Gynec Survey 15: 767, 1960.
6. Hanson, J. O.: Therapeutic donor insemination. Review of 440 cases. Calif Med 90: 130, 1959.
7. Kleegman, S. J.: Therapeutic donor insemination. Fertil Steril 5: 7, 1954.
8. Kremer, J.: A simple sperm penetration test. Int J Fertil 10: 209, 1965.
9. Potter R. G.: Artificial insemination by donors. Analysis of seven series. Fertil Steril 9: 37, 1958.
10. Ulstein, M.: Sperm penetration of cervical mucus as criterion of male fertility. Acta Obstet Gynec Scand 51: 335, 1972.
11. Whitelaw M. J.: Use of the cervical cap to increase fertility in cases of oligospermia. Fertil Steril 1: 33, 1950.

Submitted for publication Febr. 22, 1972

Magnar Ulstein
Fylkessjukehuset i Ålesund
6017 Åsestranda
Norway

Acta Obstet Gynec Scand 52: 103–108, 1973

OVARIAN CARCINOMA—A 10-YEAR SERIES FROM A PROVINCIAL HOSPITAL

J Timm

From the Departments of Obstetrics and Gynaecology (Head. Tore Wahlin), Helsingborg Hospital, Helsingborg, Sweden

Abstract 74 cases of ovarian carcinoma diagnosed and treated at the department of obstetrics and gynaecology Helsingborg hospital during the years 1956–1965 were examined with special reference to the effect of various biological, social and clinical factors on the later course. The following features were associated with more favourable prognosis younger age groups; married women; high haemoglobin values; short duration of symptoms; uterine bleeding as first symptom; low stage of tumour mucinous cystocarcinoma, and following tumour characteristics unilateral tumour unruptured tumour non-adherent tumour absence of metastases; absence of ascites. The differences regarding the 5-year survival rate were found to be statistically significant within the groups relating to age, haemoglobin value, stage and the variables given under tumour characteristics (with the exception of "unruptured tumour") The patient parity and the size of the tumour was of little or no prognostic value in the present series The prognosis was significantly better for patients who had bilateral oophorectomy combined with hysterectomy than for those who had bilateral oophorectomy alone The total 5-year survival rate was 35.1%. 6–16 years after the diagnosis 29.7% of the patients were still alive, 5.4% had died from intercurrent diseases and 64.9 from the tumour

Ovarian carcinoma is the fifth in frequency of malignant tumours in women and offers considerable diagnostic and therapeutic problems. In contrast with the other two large groups of gynaecological carcinoma, i.e. carcinoma of the uterine cervix and body the prognosis has not improved in recent years. This is mainly because of the insidious onset of ovarian carcinoma and the lack of specific early symptoms as well as its tendency to metastasize directly within the abdominal cavity with the result that in most patients the disease is not detected and treated until relatively late

Most papers on ovarian carcinoma emanate from radiotherapeutic centres, which is but natural because of the central role such units play in the treatment of this disease. The present paper concerns a 10-year series of ovarian carcinoma seen at the department of gynaecology Helsingborg hospital and is of interest to elucidate the problem as experienced by provincial hospitals with unselected primary material.

MATERIAL

The material included all cases of ovarian carcinoma diagnosed and treated at the department of gynaecology Helsingborg hospital, from 1956 through 1965 There was a total of 74 cases. This number does not include such special malignant ovarian tumours as granulosa-cell tumours or ovarian metastases from primary tumours in other organs In all cases the radiotherapy had been given at the department of radiotherapy Lund hospital. Twenty-two (30%) patients were treated only at the department of gynaecology in Helsingborg, and thus were not registered at any department of radiotherapy

The age distribution is given in Fig. 1 The distribution of the patients through the 10-year period and the number of survivors in each two-year class are given in Table 1

RESULTS

Survival

The 5-year survival rate was 35.1% i.e. 26 of the 74 patients (Fig. 2) The curve also shows the successive loss of patients during the first 5 years after diagnosis and beginning of treatment. The loss was greatest during the first year after which the curve gradually flattened off and was almost horizontal during the 4th–5th year The curve reflects, among other things, that already when first seen many of the patients had widespread

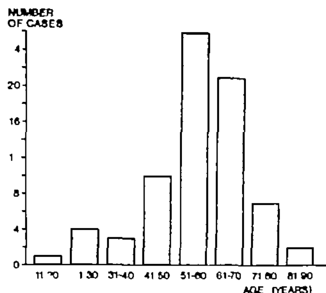


Fig 1 Age distribution of 74 patients with ovarian car cinoma diagnosed in Helsingborg 1956–65

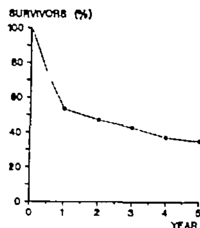


Fig 2 Survival rate in present series.

metastases with an extremely poor prognosis and that the frequency of fatal recurrences after the first year is relatively low Later however the mortality from late recurrences of the commonest sort of ovarian carcinoma, serous cystadenocar cinoma is astonishingly high an observation made in a large investigation by Aure Hoeg and Kolstad (1).

Effect of Age Civil Status and Parity on the Prognosis

The effect of age on the prognosis is clear from Table II which shows a 5-year survival rate of 55 6% for patients below 50 years, compared with 28 6% for those above this age. The dif ference is statistically significant ($\chi^2 = 3.25$ df = 1 $p < 0.05$ one-sided test)

A better 5-year survival rate for younger pa tients has also been reported by other inves tigators. Stone et al. (13) in an American series reported a 5-year survival rate of 47 3% for pa tients below 40 years and 16.1% for women above 40 years. Cutler et al. (?) gave 74 5-year survival for patients below 35 years and 28% for patients above this age.

Table II also gives the distribution of the cases among unmarried married and previously mar ried (i e. widows and divorced) and the survival rate in the respective groups. According to the table the prognosis is best for married women. But the difference is not statistically significant ($\chi = 1\ 5$ df = 2 $p > 0.05$ two-sided test) In the above mentioned material Stone et al. (13) found a survival rate of 23.5% for married women, compared with 10 3% for single women.

Table II also shows the relation between parity and prognosis. No difference was found between nulliparae and multiparae. Stone et al. (13), on the other hand, reported a lower survival rate for nulliparae (16.9% 5-year survivor rate) than for multiparae (24 3%).

Effect of Prediagnostic Duration of Symptoms on Prognosis

The figures in Table II suggest that when pa tients had symptoms for less than 3 months the further course was better than those with a

Table I *Distribution of cases of carcinoma and survival rate*

	1956–57	1958–59	1960–61	1962–63	1964–65	Total	Total
Number of patients	15	18	13	15	13	74	100
Still living	5	5	2	5	5	22	29 7
Died from carcinoma	9	11	10	10	8	48	64 9
Died from intercurrent disease[a]	1	1	2	—	—	4	5 4

The 4 patients who died from intercurrent disease died after 14 10½ 8 and 3½ years.

Table II. *Distribution of material according to age, civil status, fertility, duration of symptoms before diagnosis and further course*

	Number of patients	Survived 5 years	% survived
50 years	18	10	55.6
50 years	56	16	28.6
Unmarried	13	3	23.0
Widow, divorced	16	5	31.6
Married	45	18	40.0
Nulliparae	24	9	37.5
1-multiparae	50	17	34.0
Prediagnostic duration of symptoms 3 mo.	35	14	40.0
Prediagnostic duration of symptoms 3 mo.	28	8	28.6

longer history. But the difference was not statistically significant ($\chi^2 = 0.9$, df = 1, $p > 0.05$). The average duration of symptoms before the diagnosis in patients who survived 5 years was 3.8 months, compared with 5 months for those with shorter survival. The overall average duration of the symptoms before the condition had been diagnosed was 4.4 months.

This lack of correlation between the duration of symptoms and the further course corroborates findings in larger series (7, 13, 16). In America it has been suggested that a long duration of symptoms before diagnosis is a sign that the rate of growth of the tumour as well as its biological activity may be low. This would explain why a long prediagnostic history of ovarian carcinoma need not mean a poorer prognosis than a short one.

Symptoms

As pointed out in the introduction, ovarian carcinoma does not produce early specific symptoms. The initial symptoms are abdominal pain, abdominal swelling, bleeding and precipitant urination. The commonest symptoms in the present series and their correlation with the further course are given in Table III.

It is clear that abdominal swelling and distension were the most common initial symptoms, followed by abdominal pain, precipitant urination and bleeding, in the order given. In Gorton's (3) survey of the initial symptoms in a series of women seen at the department of radiotherapy, Lund, the commonest symptom was abdominal

Table III. *Symptoms and further course*

	Number of patients	Survived 5 years	% survival
Abdominal swelling and distension	38	13	34.2
Abdominal pain	28	9	32.1
Precipitant urination	17	6	35.3
Premenopausal bleeding 3, postmenopausal 12	15	7	46.7
Loss of weight and fatigue	14	2	14.3
Constipation	12	2	16.7

pain, followed by bleeding and abdominal swelling. In the above mentioned investigation by Stone et al. (13) the order of frequency of the initial symptoms were abdominal pain, abdominal swelling and bleeding. In our series, as in that reported by Sall et al. (11), the further course was best in those cases, where the initial symptom was uterine bleeding. It is difficult to explain why, but it is possible that the patient finds such a symptom so alarming that she seeks medical advice without delay. The outcome was worst in those patients who initially complained of general symptoms of malignant disease, such as fatigue and loss of weight, because in these the tumour has often already metastasised.

Clinical Signs and Further Course

1. *Anaemia*

Table IV shows that the course of the disease tended to vary with the haemoglobin value on admission of the patient to hospital.

A significant difference in this respect was found between patients with and without anaemia ($\chi^2 = 5.4$, df = 2, $p < 0.05$, one-sided test).

2. *Operative findings*

The prognostic value of findings at operation is clear from Tables V and VI.

The tumours were staged in accordance with

Table IV. *Haemoglobin value and further course*

	< 70	71–80	> 80
Number of patients	21	27	26
Survived 5 years	4	9	13
Per cent	19.0	33.3	50.0

Table V *Further course of the cases grouped according to stage*

	Numbers of patients	Survived 5 years	% survival
Stage I	24	18	75.0
Stage II	15	5	33.3
Stage III	18	2	11.1
Stage IV	16	1	6.3
Unexplored cases	1	0	0

Table VII. *Pathological classification*

	Number of patients	Survived 5 years	survival
Serous papillary cyst adenocarcinoma	38	16	42.1
Mucinous cystadenocarcinoma	9	6	66.7
Mixed group including unclassifiable carcinoma	27	4	14.8

the recommendations of the International Federation for Gynaecology and Obstetrics in 1971 stage I tumour confined to ovaries stage II tumour confined to pelvis stage III intraperitoneal metastases stage IV remote metastases. One case was not surgically explored As in most other series, then the more extensive the disease the worse the prognosis and the differences in 5-year survival rate between the different stages were statistically significant. Pomerance & Moltz (10) have also shown that the late prognosis—10 years—depends largely on the stage of the condition at the time of its diagnosis. Owing to differences in the principles of classification according to stage it is difficult to compare our survival rates with those in other series. However common to all series is the high percentage of cases in stages III and IV at the time of diagnosis—almost half in our series—with a consequently poor prognosis. In a series from the department of gynaecology and obstetrics, Sabbatsberg's Hospital for example 60% of the cases were in stages III and IV (4)

Table VI. *Operative findings*

(a) Size of tumour

	Number of patients	Survived 5 years	survival
Hen's egg or smaller	7	1	14.3
Goose's egg – fist	9	4	44.4
Ostrich egg or larger	57	21	36.8

(b) Other findings

	Presence of symptoms		Absence of symptoms	
	Number of patients	Survived 5 years (%)	Number of patients	Survived 5 years (%)
Bilateral ovarian involvement	33	15.2	39	53.8
Ascites	40	17.5	33	57.6
Metastases	42	11.9	30	70.0
Adhesion	36	16.7	38	52.6
Tumour ruptured	33	6.1	30	40.0

Estimation of the size of the tumour proved of no prognostic value the subgroups were too small to warrant any conclusion As with all malignant tumours of the abdomen ascites, metastases bilateral involvement and adhesion of the tumour to surrounding tissues were signs of a poor prognosis. As expected the prognosis was poorest for those with metastases—only 11.9% of such patients survived 5 years.

When the tumour ruptured before or at operation the prognosis was worse (Table VI) The difference in survival between patients with unruptured and ruptured tumours was, however not statistically significant ($\chi^2 = 1.3$ $df = 1$ $p < 0.05$ two-sided test).

In an American series Turner Remine & Dockerty (1959) found that more than 50% of the patients in whom the tumour had ruptured before or at operation survived more than 5 years, a figure suggesting that rupture is of negligible prognostic significance. The authors based their conclusion on 42 cases They claimed that the factor deciding the prognosis also in patients with a ruptured tumour is the clinical stage of the lesion. Other American authors (Munnell (7) and others) have published similar results. However recent investigations (Müller Kolstad, Wolff) suggest that the prognosis is worse if the tumour has ruptured. According to Kottmeier rupture worsens the prognosis of a serous papillary cystadenocarcinoma but it is doubtful

Table VIII. *Treatment*

	Number of patients	Survived 5 years	% Survival
No treatment (only surgical exploration)	8	0	0
Only radiotherapy (palliative)	4	0	0
Only surgical treatment	14	6	42.9
Unilat salpingo-oophorectomy	4	1	25.0
Bilat salpingo-oophorectomy	8	4	50.0
Bilat salpingo-oophorectomy hysterectomy	2	1	50.0
Surgical treatment radiotherapy	48	20	41.7
Unilat salpingo-oophorectomy	—	—	—
Bilat salpingo-oophorectomy	32	9	28.1
Bilat salpingo-oophorectomy hysterectomy	16	11	68.8

bether rupture has such an effect on mucinous cystadenocarcinoma (Kottmeier personal communication).

Patho-anatomical Classification

The tumours were examined histologically at the Institute of pathology in Lund (Head: C. G. Ahlström) and the results are given in Table VII. Classification of the material was difficult because of the changes in principles of classification during the period covered. All of the histological descriptions of the operative specimens were therefore carefully analysed and the terminology used in the pathologists' reports was made as uniform as possible.

The frequency of so-called unclassifiable tumours, very advanced tumours where only diagnostic biopsy specimens had been obtained and where the form of tumour could not be determined histologically with certainty, was fairly high. These cases were assigned to a "mixed group" together with a number of cases of poorly differentiated, partly necrotic and disintegrating cystadenocarcinoma, anaplastic carcinoma and a number of cases of probably endometrioid tumours. This group was thus very heterogeneous; it was used to get the other groups as homogeneous as possible. It is clear from the table that serous papillary cystadenocarcinoma was the commonest histological type. Investigations of large series have shown this tumour to have a very poor prognosis—Kjellgren (5) gives 20% 5-year survival rate—and in the vast majority of the patients with this type of carcinoma surgical exploration reveals metastases outside the pelvis (6).

Treatment

The surgical treatment given is summarised in Table VIII. 14 patients received surgical treatment only, 48 both surgery and radiotherapy with a therapeutic dose. The remaining 12 patients had widespread metastases and in these the prognosis was considered very poor: 4 of them were given palliative radiotherapy while 8 were subjected only to surgical exploration and diagnostic biopsy. The reason why 14 patients received surgery alone was either because the lesions were only local and regarded as readily accessible to radical surgery (6 cases) or because the changes were very advanced and the patients were in a very poor general condition (8 cases).

Routine treatment consisted of bilateral oophorectomy with or without hysterectomy and postoperative radiotherapy, usually in the form of intrauterine or intravaginal radium and external roentgen therapy. The radiation doses varied from case to case depending on the patients' general condition, age, extent of the disease and other factors. A few of the patients received radiotherapy only preoperatively. A few of the patients were treated with radioactive gold (Au^{198}) intraperitoneally; some were treated only with radium and gold. Nine patients received supplementary treatment with Sendoxan. These patients had metastases already at the time of diagnosis and the operation had not been radical, or recurrences had soon appeared. Eight of these patients died within 5 years and one after 5½ years.

A comparison was made between the group of patients treated surgically with bilateral oophorectomy only and the group which was subjected also to hysterectomy; both groups were given postoperative radiation therapy (χ^2-test according to Siegel 12). 32 patients were treated by bilateral oophorectomy. Nine of these, 28.1%, survived 5 years. Sixteen were treated by bilateral oophorectomy + hysterectomy, 11 of whom survived 5 years (68.8%). The difference was significant at

Table V. *Further course of the cases grouped according to stage*

	Numbers of patients	Survived 5 years	% survival
Stage I	24	18	75.0
Stage II	15	5	33.3
Stage III	18	2	11.1
Stage IV	16	1	6.3
Unexplored cases	1	0	0

Table VII. *Pathological classification*

	Number of patients	Survived 5 years	% survival
Serous papillary cyst adenocarcinoma	38	16	42.1
Mucinous cystadenocarcinoma	9	6	66.7
Mixed group including unclassifiable carcinoma	27	4	14.8

the recommendations of the International Federation for Gynaecology and Obstetrics in 1971: stage I, tumour confined to ovaries; stage II, tumour confined to pelvis; stage III, intraperitoneal metastases; stage IV, remote metastases. One case was not surgically explored. As in most other series, then, the more extensive the disease the worse the prognosis, and the differences in 5-year survival rate between the different stages were statistically significant. Pomerance & Moltz (10) have also shown that the late prognosis—10 years—depends largely on the stage of the condition at the time of its diagnosis. Owing to differences in the principles of classification according to stage, it is difficult to compare our

Table VI. *Operative findings*

(*a*) *Size of tumour*

	Number of patients	Survived 5 years	% survival
Hen's egg or smaller	7	1	14.3
Goose's egg – fist	9	4	44.4
Ostrich's egg or larger	57	21	36.8

(*b*) *Other findings*

	Presence of symptoms		Absence of symptoms	
	Number of patients	Survived 5 years (%)	Number of patients	Survived 5 years (%)
Bilateral ovarian involvement	33	15.2	39	53.8
Ascites	40	17.5	33	57.6
Metastases	42	11.9	30	70.0
Adhesion	36	16.7	38	52.6
Tumour ruptured	23	26.1	50	40.0

survival rates with those in other series. However, common to all series is the high percentage of cases in stages III and IV at the time of diagnosis—almost half in our series—with a consequently poor prognosis. In a series from the department of gynaecology and obstetrics, Sabbatsberg's Hospital, for example, 60 % of the cases were in stages III and IV (4).

Estimation of the size of the tumour proved of no prognostic value; the subgroups were too small to warrant any conclusion. As with all malignant tumours of the abdomen, ascites, metastases, bilateral involvement and adhesion of the tumour to surrounding tissues were signs of a poor prognosis. As expected, the prognosis was poorest for those with metastases—only 11.9 % of such patients survived 5 years.

When the tumour ruptured before or at operation, the prognosis was worse (Table VI). The difference in survival between patients with unruptured and ruptured tumours was, however, not statistically significant ($\chi^2 = 1.3$, df = 1, $p < 0.05$, two-sided test).

In an American series Turner, Remine & Dockerty (1959) found that more than 50 % of the patients in whom the tumour had ruptured before or at operation survived more than 5 years, a figure suggesting that rupture is of negligible prognostic significance. The authors based their conclusion on 42 cases. They claimed that the factor deciding the prognosis also in patients with a ruptured tumour is the clinical stage of the lesion. Other American authors (Munnel (7) and others) have published similar results. However, recent investigations (Muller, Kolstad, Wolff) suggest that the prognosis is worse if the tumour has ruptured. According to Kottmeier rupture worsens the prognosis of a serous papillary cystadenocarcinoma, but it is doubtful

Acta Obstet Gynec Scand 52 109–112, 1973

RADIOISOTOPE RENOGRAPHY IN THE SURGICAL MANAGEMENT OF CARCINOMA OF THE CERVIX

P Mogensen, P Rødbro and H Lefèvre

From the Department of Clinical Physiology (Heads: O Munck & J Giese), the Department of Gynecology and Obstetrics (Heads: H. Lefèvre, J Weber & E. Never) and the Department of Radiology (Heads: H Elsner, M Egeblad & P Ahlgren), Glostrup Hospital, Glostrup Denmark

Abstract The results of intravenous pyelography (IVP) and radioisotope renography (RNG) were compared pre- and postoperatively in 77 patients with carcinoma of the cervix. A comparison was made of the occurrence of obstructive uropathy. A total of 161 comparisons between the two tests were done. The results revealed that the IVP never was abnormal without the RNG also being so. The data suggest that radioisotope renography to a large extent can replace intravenous pyelography in the evaluation of gynecological patients after operation.

It is important to study urinary tract function in patients with carcinoma of the cervix (CC) before and after treatment (1, 6, 14). The presence of ureteric obstruction prior to treatment indicates a bad prognosis. If it follows immediately after operative treatment for CC, it may indicate operative injury and if ureteric obstruction comes on gradually after operative treatment it may be a sign of recurrence.

Intravenous pyelography (IVP) has for long been the established procedure for this investigation. IVP does not only visualize obstruction caused by the disease itself and its treatment, but may also show malformations of the urinary tract. It is of less value in the diagnosis of other post-operative complications such as fistulas and atonic bladder where however the clinical course combined with other procedures are informative (6, 7).

Radioisotope renography (RNG) has been proposed as a valuable supplement to pre- and post-operative investigations in CC patients (4, 5, 14). Though the RNG is non-specific and offers no information about morphology, it embodies in its curve pattern much of the information hitherto obtainable only by IVP. Furthermore RNG yields information about individual kidney function and exceeds IVP for this purpose (11). This is valuable in the treatment of obstructive uropathy since repeated renograms can determine whether the function of an obstructed kidney is decreasing or constant.

In a series of CC patients we have compared the outcome of pre- and postoperative IVPs with RNG. The comparison was done with regard to the occurrence of obstructive uropathy. The purpose was to elucidate whether or to what extent, RNG can replace IVP in the surgical management of CC patients.

PATIENTS AND METHODS

Patients

This is a consecutive series of 77 patients (age between 29 and 58 years) with carcinoma of the cervix, in whom operative treatment was indicated. Twenty-six patients had stage I cervical cancer. In these patients a radical Wertheim hysterectomy was performed as described by Meigs (10), with dissection of the pelvic lymph nodes and removal of the paravaginal connective tissue and the proximal one third of vagina. The vascularized ureteric sheath was preserved by Palmrich's method (13), and an attempt was made to preserve the roof of the ureter as described by Novak (12). Carcinoma in situ of cervix uteri was found in 51 cases. These patients had a modified version of the hysterectomy described above, as the lymph nodes and the paravaginal connective tissue were not removed. During the operation in two patients* a ureter was accidentally cut close to the bladder but was successfully reimplanted into the bladder. In one patient an-

* These two patients belonged to the initial group of the series. The accidents occurred in connection with amputation of the upper part of the vagina, a procedure which at this early moment was done without laying the ureters bare. The accidents led to the operation described above.

the 2%-level ($\chi^2 = 6.3$ df = 1 $0.02 < p < 0.01$ two-sided test) Supplementary hysterectomy thus improved the late results.

DISCUSSION

Three of the patients in the present series had an ovarian cystoma suspected of being malig nant. At after-examination of the preparations (Prof C. G Ahlström) these tumours were as signed to groups 1 B 1 B and 2 B respectively and still regarded as probably malignant. It is thus doubtful whether these 3 cases really should be regarded as examples of ovarian carcinoma. If they are not included as such the 5-year survival rate is 32.4% instead of 35 1%

Our 5-year survival rate was somewhat higher than that reported in published series. Wetterdal (15) for example gave 18% for a series from Sabbatsberg Hospital (1959). Stone et al (13) reported 20 6% and the corresponding figure of 6 697 cases from The US National Cancer In stitute (1961) was 23 8% Van Orden et al. (9) gave 23% for another American series. The best 5-year cure rate 54 3% was reported by Turner Remine & Dockerty (14) from the Mayo Clinic in Rochester However their series is not com parable to ours because it included only operated cases (55% received also postoperative radio-therapy)

The relatively high 5-year survival rate in the Helsingborg series may perhaps be explained by the high percentage of patients—almost one third—with carcinoma stage I

No satisfactory explanation can be offered for the more favourable prognosis of younger pa tients. However it has been tentatively ascribed to a better general condition and better healing conditions, as well as a more favourable hor monal status up to the menopause i.e. a better defence against ovarian carcinoma, which is most common after the menopause.

The very poor outcome for patients in which the tumours were smallest—only 1 (14 3%) of 7 patients survived 5 years (see Table VI *a*)—is remarkable It was found that in the remaining 6 patients in this group by the time of operation the tumour had already metastasised. This was probably due to the biological activity of the tu-mours.

REFERENCES

1 Aure, J C., Hoeg, K. & Kolstad, P Obstet Gynec *37* 1 1971
2. Cutler S. J Ederer F G. & Greenberg, K. A J Nat Cancer Inst *24* 541 1960.
3 Gorton, G S Läkartidn *49* 2931 1951
4 Ingelman-Sundberg, A. Sv Läkartidn *64* 4037 1967
5 Kjellgren, O., Gynekologisk cancer Almqvist & Wik sell, Stockholm, 1967
6 Kottmeier H L. Amer J Roentgen *111* 417 1971
7 Munnel, E. W Cancer *5* 1128 1952.
8 National Cancer Institute End Result and Mortality Trends in Cancer US Govt. Print. Office Washington DC, 1961
9 Orden, D E. van et al. Amer J Obstet Gynec *94* 195 1966.
10. Pomerance W & Moltz, A. Abstracts of Tenth International Cancer Congress, Houston, Texas, 1970.
11 Sall, S et al., Surg Gynec Obstet *117* 601 1963.
12. Siegel, S Nonparametric Statistics for the Be-havioral Sciences. McGraw Hill, New York, 1956.
13 Stone M. C et al. Surg Gynec Obstet *116* 351 1963
14 Turner J C Remine W H. & Dockerty M. B Surg Gynec Obstet *109* 198, 1959
15 Wetterdal, P S Läkartidn *58* 2299 1961
16. Wheeler F G Fennell, R. N & Meigs, J V New Engl J Med *45* 447 1951

Submitted for publication March 3 1972

Jurgen Timm
Department f Obstetrics and Gynaecology
Helsingborg Hospital
251 87 Helsingborg
S eden

Table I. *Preoperative comparison between results of radioisotope renography (RNG) and intravenous pyelography (IVP) in 77 patients (77 investigations)*

N Normal, *a*, borderline, *A*, abnormal. Figures in italics denote agreement between RNG and IVP

IVP	RNG *NN*	*Na*	*aa*	*NA*
NN	*64*	4	1	0
Na	4	*0*	0	2
aa	2	0	*0*	0

as the RNG was more pathological than the IVP or vice versa.

In 14 cases the IVP was the more pathological, the IVP/RNG relations being: *Na*/*NN* (6 cases), *aa*/*NN* (7 cases) and *aa*/*Na* (1 case). The diuresis during RNG in all these cases was 9 ml per min or more.

In 31 cases the RNG was the more pathological, the IVP/RNG relations being: *NN*/*Na* (11 cases), *NN*/*aa* (7 cases), *NN*/*NA* (3 cases), *NN*/*aA* (1 case), *NN*/*AA* (1 case), *N*/*NA* (4 cases), *aa*/*aA* (1 case), *aa*/*AA* (1 case), *Na*/*aA* (1 case) and *aA*/*AA* (1 case). The diuresis during RNG was below 1 ml per min in 8 of these 31 cases.

All renograms except two from the above mentioned patient with ureteric stricture returned to normal within the observation period (maximum 6 weeks).

DISCUSSION

RNG is generally considered more sensitive than IVP in disclosing minor renal excretory abnormalities (2, 3, 15). In 67 CC patients after hysterectomy Drache et al. (2) found an abnormal RNO in 24 patients, but only seven of these had an abnormal IVP. These authors, however, did not measure the diuresis during RNG. The time of appearance of maximal activity (t_m) and the residual activity (A_{20}) both increase as the urine flow decreases (17). Thus, in order not to get too many "falsely pathological" renograms, due to the inbuilt high sensitivity of the test, sufficiently high diuresis should be aimed at. In our experience this means that the diuresis should exceed 2 ml per min. The importance of strictly adhering to a rigid standard procedure when performing RNG has been stressed by other workers (16).

Table II. *Postoperative comparison between results of radioisotope renography (RNG) and intravenous pyelography (IVP) in 77 patients (84 investigations)*

N Normal; *a*, borderline; *A*, abnormal. Figures in italics denote agreement between RNG and IVP

IVP	RNG *NN*	*Na*	*aa*	*NA*	*AA*	*aA*
NN	*39*	7	6	3	1	1
Na	2	*4*	0	2	0	1
aa	5	1	*2*	0	1	1
NA	0	0	0	*5*	0	0
AA	0	0	0	0	*2*	0
aA	0	0	0	0	1	*0*

In itself radical hysterectomy, because of postoperative oedema, leads to transitory dilatation of the renal calyces; unless infection occurs no treatment is necessary for this condition, since it regresses gradually within four to eight weeks (6, 7). This is reflected by the number of borderline pyelograms and borderline or abnormal renograms postoperatively in our series (Table II). Had the RNG been done earlier in the postoperative period the incidence of abnormal renograms probably would have been greater. On the other hand, persistent renal damage may follow ureteric obstruction lasting about 12 days (9). It is therefore appropriate to do the RNG about the 10th day after operation.

We recommend the following schedule before and after operative treatment of CC. *Preoperatively* a RNG should be done. If this—with diuresis above 2 ml per min—is dubious or abnormal, an IVP should be carried out. *Post-*

Table III. *Summary of 161 pre- and postoperative comparisons between results of radioisotope renography (RNG) and intravenous pyelography (IVP) in 77 patients*

N Normal, *a*, borderline, *A*, abnormal

	RNG not abnormal on any side (*NN*, *Na*, *aa*)	RNG abnormal on one or both sides (*NA*, *AA*, *aA*)
IVP not abnormal on any side (*NN*, *Na*, *aa*)	141	12
IVP abnormal on one or both sides (*NA*, *AA*, *aA*)	0	8

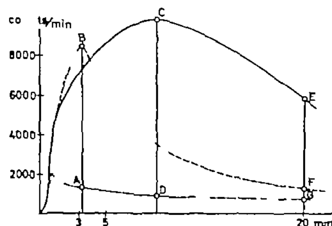


Fig 1 Typical renographic findings in patient with normal function of right kidney: t (the time interval from injection to maximal renal activity) = 3.– min. A_{m} (residual renal activity 20 min after injection in per cent of maximal renal activity (both corrected for background activity)) = FG/BA = 8% and obstructive uropathy of left kidney (t_{m} = 8.8 min, A_{m} = EG/CD = 58%). According to criteria (see text) renogram from right kidney classified as *normal*, from left kidney as *abnormal* Ordinate: recorded activity Abscissa time (min) Right kidney — left kidney· background.

lateral ureteric stricture following hysterectomy had to be treated operatively None of the patients received X-ray treatment during the study

Radioisotope renography

The RNG was done with the patient sitting in chair after she had been given one litre of water to drink. Sodium ortho-iodohippurate labelled with ^{131}I (^{131}I-Hippuran) in a dose of 0.2 µCi per kg body weight was injected intravenously The activity over each kidney region and over the left scapula was followed for 20 minutes with scintillation detectors, the scapula curve recorded the background activity The detailed renography procedure is described elsewhere (11). As the renographic abnormalities following operation usually consist of a prolonged accumulation phase or a prolonged excretion phase r both (5), two parameters were calculated from each renogram (Fig. 1) t_{m} the time interval from injection to maximal renal activity A_{m} residual renal activity 20 min after injection as a percentage of maximal renal activity (both corrected for background activity) t_{m} and A_{m} were determined also in 39 control subjects, who were normotensive and without urological symptoms. In these subjects the mean value, standard deviation, and upper limit of the 99% confidence interval were for t 2.6 min, 0.7 min, and 4.4 min, respectively For A_{m} the corresponding figures were 15% 6% and 30.– The average diuresis in the control group was 7.6 ml per min (range 1.4–15.0 ml per min).

Each renogram from the patients was classified into one of three categories:

1 *Normal (N)*: $t < 4.4$ min *and* $A_{m} < 30\%$
2. *Borderline* (a) *Either* $t_{m} > 4.4$ min *or* $A_{m} > 30\%$
3. *Abnormal (A)* $t_{m} > 4.4$ min *and* $A_{m} > 30\%$

Intravenous pyelography

After the usual bowel preparation the IVP was performed with injection of 20 ml 76% sodium and meglumine amidotrizoas and pictures taken 5 10, 15 and 30 min after the injection. Without knowledge of the RNG the pyelograms were classified into one of three categories:

1 *Normal (N)*: Normal pyelogram without dilatation of renal pelvis or ureter

– *Borderline* ( ): Slight dilatation of renal pelvis or ureter without stasis.

3 *Abnormal (A)* Pronounced dilatation of renal pelvis and ureter with stasis.

Plan of investigation

The RNG was done shortly before operation and approximately 10 days after operation. If not normal, the RNG was repeated after one week, and later on by intervals of two to three weeks until the RNG was normal.

The IVP was done preoperatively and 7–10 days after operation. If abnormal the IVP was repeated after a further 14 days. IVP and RNG were never done on the same day but as closely as possible Only simultaneous investigations were included in the study (i.e. here IVP and RNG were done within four days). This left, in the 77 patients, 77 preoperative and 84 postoperative comparisons between RNG and IVP The mean diuresis during the RNG was 6.5 ml per min in the preoperative series (range 0.3–16.0 ml per min) and 5.0 ml per min in the postoperati series (range 0.1 11.– ml per min).

Abbreviations

In the following the abbreviations *N* (normal) *a* (borderline) and *A* (abnormal) are used according to the above mentioned criteria for IVP and RNG. Thus *Na* means that the renogram (or the pyelogram) on one side was *normal* on the other side *borderline* *AA* means that the renogram (or the pyelogram) was *abnormal* on both sides.

RESULTS

The results of the 77 preoperative investigations are given in Table I and the results of the 84 postoperative investigations in Table II Thus a total of 161 comparisons between IVP and RNG were done the salient features of which are summarized in Table III in this table "normal" and borderline" were compiled into one group for both IVP and RNG In twelve cases with abnormal RNG the IVP was normal or borderline. The IVP was never abnormal without the RNG also being so.

In 116 comparisons the results of RNG and IVP were in agreement (figures in italics in Tables I and II). In 45 cases the results differed

Acta Obstet Gynec Scand 52, 113–130 1973

THE CLINICAL VALUE OF AMNIOTIC FLUID ANALYSIS IN PREGNANCIES COMPLICATED BY RH ISOIMMUNIZATION OR HEPATOSIS

Lars Erik Jonasson

From the Department of Obstetrics and Gynaecology (Prof. Carl Gemzell), University of Uppsala, Uppsala, Sweden

Abstract. 605 samples of amniotic fluid were obtained by abdominal amniocentesis in 363 late pregnancies. The liquor was analysed with respect to ΔE_{450} (the optical density at 450 nm), progesterone and total protein content. On 177 samples measurements of the acid and alkaline phosphatase activity were also performed. Most of the samples came from pregnancies complicated by isoimmunization, but the material also contained samples from women with hepatosis gravidarum or other complications as well as from normal pregnancies. The measurements have been studied in relation to duration of pregnancy, cord blood haemoglobin levels, cord serum bilirubin levels, infant birth weight, maternal serum bilirubin levels, maternal alkaline phosphatase levels, maternal age, order of pregnancy, maternal and infant blood groups. For part of the series the relationships between the parameters were studied by multiple regression analysis. The results indicate a rather weak correlation between infant cord blood haemoglobin levels and the other parameters. The ABO blood groups of mother and infant influence the levels of cord blood haemoglobin, cord serum bilirubin and amniotic fluid total protein. Amniotic fluid progesterone and ΔE_{450} values are correlated to amniotic fluid total protein levels. Low ΔE_{450} values indicate that the fetus is mature. Determinations of ΔE_{450} before the 37th week show a better correlation with the condition of the infant than later ones. Order of pregnancy influences the total protein content of amniotic fluid in normal pregnancies. Determinations of acid and alkaline phosphatases in amniotic fluid do not give any clinically important information in cases of isoimmunization. Meconium contamination strongly increases alkaline phosphatase activity. Attempts to use the rapid method of Brem for oestrogen determinations in amniotic fluid proved to be difficult for technical reasons.

Estimation of bile pigments in the amniotic fluid now has an accepted role in the clinical management of isoimmunization in pregnancy. This method was introduced by Bevis about 20 years ago (4, 5) and its clinical application was further developed by Walker (42) and Liley (27, 28). Since then a large number of clinical studies have been published dealing with this subject. Most studies have been concerned with various modifications of the methods for determination of the amniotic fluid bilirubin content and the proper interpretation of the findings.

Since 1965 spectrophotometric analysis of amniotic fluid according to Liley has been used in this department as a tool in the routine management of isoimmunization in pregnancy. The liquor amnii has also been examined with respect to a number of other parameters and his study is an attempt to evaluate these findings from a clinical standpoint.

MATERIAL

In the majority of cases samples of amniotic fluid were taken by abdominal amniocentesis. Some samples were taken by direct uterine puncture at the time of elective caesarean section. The technique of amniocentesis was described previously (21). None of the patients were in labour. A total of 605 samples from 363 women in the last trimester of pregnancy were obtained. After amniocentesis a sudden increase in maternal antibody titres of at least two dilution steps was observed in 16 out of 239 patients with isoimmunization and affected infants. In one patient premature separation of the placenta occurred during labour the day after amniocentesis. No other complications of amniocentesis were observed.

Composition of the total series

A. *Normal pregnancies*

(*a*) Patients with contracted pelvis but otherwise normal pregnancies. Samples taken at the time of elective caesarean section. 20 samples from 20 patients.

(*b*) Rh-immunized patients whose infants were Rh negative and healthy. 81 samples from 48 patients.

(*c*) Patients with immunization to other blood group systems. Infants negative for the involved blood antigen and healthy. 20 samples from 13 patients.

6 731543

operatively RNG is performed on the 10th day a normal renogram safely rules out ureteric obstruction if the renogram is dubious, the patient is controlled by weekly RNG should the renogram on the 10th day (or later) be abnormal an IVP should be performed.

Applying this programme in the present series: only 7 out of 77 preoperative IVPs would have been done Of the 77 postoperative renograms around the 10th day 45 were normal 16 were borderline, and 16 were abnormal on one or both sides. Thus only 16 of 77 postoperative IVPs would have been carried out.

The RNG of course yields no anatomical information A more conservative conclusion from the present data would therefore be to recommend an IVP *and* a RNG before operation and then postoperatively to follow the patient with RNG as described above.

The demands for urological investigations are less in operations for benign gynecological disorders. In these conditions it has been questioned whether the information obtained by IVP *prior* to operation is strictly necessary (8). In such conditions also the RNG may be of considerable value.

^{131}I Hippuran renography is a simple and safe method to evaluate important aspects of renal and ureteric function It has several advantages over IVP: The patient does not find it unpleasant and requires no preparation the radiation dose is very small, making it ideally suited for serial studies it is *less time-consuming* and *it is significantly cheaper* Our results warrant the conclusion that radioisotope renography to a large extent may replace intravenous pyelography in the evaluation of gynecological patients after operation.

REFERENCES

1 Burns, B. C., Everett, H. S. & Brack, C. B Value of urologic study in the management of carcinoma of the cervix. Am J Obstet Gynec *80* 997 1960.

2 Dische S., Caplan, L. & Kramer S. The isotope renogram in carcinoma of the cervix. Am J Roentgenol Radium Ther Nucl Med 90 149 1963.

3 Erez, S., Jordan, W M Kaplan, A. L. & Burdine J A. Use of radioisotope renography and intravenous pyelography in carcinoma of the cervix. Am J Obstet Gynec 97 947 1968

4 Gerbie A. B., Flanagan, C. L. & Woodbury L P Pre- and postoperative ureteral dysfunction. Am J Obstet Gynec *83* 1138 196_

5 Gerbie A. B. & Flanagan, C. L. Gynaecologic applications of the radioisotope renogram. Am J Obstet Gynec *84* 1838 1962.

6 Green, T H., Meigs, J V Ulfelder H. & Curtin, R. R.. Urologic complications of radical Wertheim hysterectomy: incidence, etiology management and prevention. J Obstet Gynec *70* 29 196_

7 Hanafee, W Ottoman, R. E. & Wilk, S. P Roentgen diagnosis of urinary complications following radical hysterectomy and pelvic lymph node dissection. Radiology *70* 46, 1958.

8. Hartog, E. M. & O'Leary J A.. The value of routine intravenous pyelography prior to elective gynecologic operations. J Fla Med Assoc *56*. 790, 1969

9 Katul, M J & Wax, S. H.. Evaluation of renal function during experimental hydronephrosis by means of the radioisotope renogram. Surg Gynec Obstet *1*?? 563 1968.

10. Meigs, J V Radical hysterectomy for cancer of the cervix with bilateral pelvic lymph node dissection *In Operative Technic in Specialty Surgery* (ed. Warren H Cole). Appleton-Century-Crofts, New York, 19??

11 Mogensen, P Rossing, N & Giese J Glomerular filtration rate measurement and ^{131}I-Hippuran renography before unilateral nephrectomy Scand J Urol Nephrol 6. 228, 1972.

12. Novak, Franc The prevention of uretero-vaginal fistulas after the Wertheim operation. Int J Gynec Obstet *7* 301 1969

13 Palmrich, A. H Die Methode der Wertheim-Operation mit Lymphknotenentfernung an der I. Universitäts-Frauenklinik Wien. Geburtsh Frauenheilk *?* 829 1961

14 Roddick, J W Gerbie A. B. & Flanagan, C. L. The use of the *radioisotope renogram in* the follow-up of treated gynaecologic malignancy Am J Obstet Gynec *88* 97 1964

15 Serratto, M Grayhack, J T & Earle D. P A clinical evaluation of the iodopyracet (Diodrast) renogram. Arch Int Med *103* 851 1959

16. Wax, S H. & M Donald, D. F The renogram vs. pyelogram: Evaluation of the significance of upper urinary tract obstruction. J Urol 96 816, 1966.

17 Wedeen, R. P Goldstein, M H. & Levitt, M. F The radioisotope renogram in normal subjects. Am J Med *34* 765 1963

Submitted for publication May 18 1972

P Mogensen
Department of Obstetrics and Gynecology
Glostrup Hospital
Copenhagen
Denmark

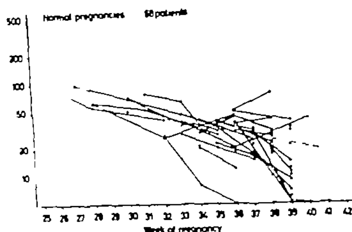


Fig. 1. CI values in normal pregnancies. The broken lines indicate the Liley zones.

carefully by skilled technicians the results are reproducible. Recovery experiments were made using a pool of amniotic fluid containing 0.160 µg oestrogen/ml. Determinations on the unfrozen pool ranged from 0.154 to 0.166 µg/ml (n 4), and on the frozen pool from 0.158 to 0.176 µg/ml (n 4). The addition of oestriol 0.200 µg/ml gave recovery of 46%, 0.400 µg/ml 45%, 0.900 µg/ml 73% and 1.500 µg/ml 72% (mean of 4 determinations each). The coefficient of variation for 13 unfrozen samples with double determinations was 6.9%. The range for these samples was 0.072–0.970 µg/ml. These samples were kept in refrigerator at +4 to 6°C for 30 to 45 days between the determinations. The storing of unfrozen samples for that length of time thus had little effect upon the oestrogen content. The coefficient of variation when comparing determinations of unfrozen and frozen samples was 7.2% (n 8).

On 172 consecutive samples from 110 pregnancies determinations of alkaline and acid phosphatases were performed according to the method described by Beckman (2) and Bachman et al (3). 101 of these samples were from pregnancies with complete data on the mother and infants and this series was subjected to stepwise regression analysis (13), program BMD 02 R (6). A detailed description of the phosphatase analysis and the results in normal pregnancies will be published elsewhere.

RESULTS

The following expressions will be used to denote statistical significance: Not significant ($P > 0.05$), probably significant ($0.05 > P > 0.01$), significant ($0.01 > P > 0.001$) and highly significant ($0.001 > P$).

CI Levels

A. Normal pregnancies

The individual CI values showed a rather wide scatter which was especially pronounced during the last few weeks of pregnancy. The majority of values fell in the lowest Liley zone (Fig. 1). The borders of the Liley main zones are marked in the figures. In 23 patients repeated samples were taken and 5 of these showed increasing values.

The mean values followed rather closely the border line between the lower and the middle zones up to the 37th–38th week. From then on there was a more rapid decrease (Fig. 6, Table I).

B. Cases of Rh-Isoimmunization

Group I. The individual values showed the same pattern as in the normal pregnancies. The CI values were mostly found in the lower zone or in the lower part of the middle zone (Fig. 2). Only two of 20 patients had an increase in values at repeated sampling.

The mean values were in the lower part of the middle zone and declined with advancing gestational age (Fig. 6). When the mean values were compared with the means from normal pregnancies a probably significant difference was found for the weeks 33–34 and 35–36. Later in pregnancy the differences were not significant (Table I).

Group II. The majority of the individual values fell in the lower part of the middle zone (Fig. 3). Increasing values were noted in 8 of 38 patients with repeated sampling.

The mean values were located centrally in the middle zone and showed the same sharp decline after the 37th–38th week as the normal pregnancies (Fig. 6). The mean values were only slig-

Patients belonging to the categories mentioned above were considered to represent normal pregnancies. This was perhaps not strictly true as the majority of them had shown actual isoimmunization in previous pregnancies but it was not considered justifiable to carry out amniocentesis without a clinical indication in nonimmunized mothers. All the infants of isoimmunized mothers which were regarded as "unaffected" had negative direct Coomb's tests on cord blood.

B. Patients with isoimmunization and ffected fetuses

( ) Patients with Rh-isoimmunization. 436 samples from _37 patients. These pregnancies were further classified according to the condition of the infants.

Group I Pregnancies which resulted in babies with slight haemolytic disease. Cord blood haemoglobin 1_1 g/100 ml or higher At most one exchange transfusion. 8 pregnancies.

Group II Pregnancies complicated by moderate haemolytic disease. Infant cord blood haemoglobin 8 1-1_0 g/100 ml. At most three exchange transfusions. This group also included babies with higher haemoglobin values who required more than one exchange transfusion. 69 pregnancies.

Group III Pregnancies with severe haemolytic disease. Infant cord blood haemoglobin 8.0 g/100 ml or lower This group also included babies who required more than three exchange transfusions. 56 pregnancies.

Group IV Pregnancies with ery severe haemolytic disease resulting in fetal prenatal or postnatal death. This group consisted of 30 pregnancies but in 9 of these postnatal complications was considered to be the main cause of infant death.

(*b*) Patients with isoimmunization in other blood group systems Four samples from tw patients. Only one infant needed single exchange transfusion.

The infants were counted as affected by haemolytic disease if they were positive for the invol ed blood antigen and if the direct Coomb's test performed on cord blood was positive.

C. P tients ith hepatosis gravidarum

Pregnancies not complicated by isoimmunization. The diagnosis used here w synonymous with benign idiopathic cholestasis of pregnancy (17 19) 30 samples from ?9 patients.

D Nonimm nized patients ith other complications f pregnancy

14 samples from 14 patients.

When maternal antibody titres or previous obstetrical history indicated a risk of serious fetal haemolytic disease amniocentesis was first done in the _5th-28th week of pregnancy and then repeated weekly every weeks. In cases with prediction f slight to moderate disease the first amniocentesis was done in the 3_nd-35th week and then repeated every weeks. Many exceptions occurred to these rules as patients often were referred lat to the department. During the period covered by this study the indications for amniocentesis were kept wide and only in cases with slight isoimmunization reported near term was amniocentesis sometimes omitted, as the result would not have influenced the management. series was thus not quite complete for the investiga period with respect to cases of isoimmunization, but exceptions were few

The duration of the pregnancies was calculated the first day of the last menstrual period. Patients w uncertain menstrual data were excluded from furth study Samples contaminated by visible amounts of b or meconium were also discarded. Incomplete m also caused exclusion of samples. The number of sampl remaining in the various clinical groups is indicated in tables of results which were used for the statistical analysis.

METHODS

After amniocentesis the amniotic fluid was treated a described previously (21). Determinations of ΔE_{450} were made according to the method described by Liley (2 To get whole figures the values were multiplied by 1000. This product was called Colour Index (CI). The volume of fluid necessary for one determination was 5 ml but usually about 30 ml were aspirated. The readings were mostly made on an automatic spectrophotometer and the whole spectral absorption curve between the wavelengths 350 and 700 nm was always recorded. The determinations were done immediately after sampling and the samples were kept in the dark to avoid degradation of bilirubin. Parts of the samples not needed for the spectrophotometric readings were used for other examinations.

In most cases samples of maternal venous blood were collected on the same day as the amniocentesis. All patients had their blood groups determined (AB0 and Rh systems) and were screened for antibodies by the routine methods of the hospital. In all cases of isoimmunization the maternal antibody titres were determined at regular intervals (papain method, indirect Coomb's test). All patients with isoimmunization and all patients with hepatosis gravidarum were followed by routine liver function tests (bilirubin, thymol turbidity alkaline phosphatases, transaminases in serum).

All infants born to isoimmunized mothers were blood grouped and the direct Coomb test was made on their cord blood. Determination of the cord blood haemoglobin were made by the routine methods of the hospital (cyanomethaemoglobin). The cord serum bilirubin concentrations were determined by routine method (30) The hospital paediatricians xamined and treated all the babies of isoimmunized mothers The indications for exchange transfusions were unchanged during the period covered by this study

Determinations f the total protein and the pregesterone content of amniotic fluid ere made as described previously (20 _3). The rapid method f Brown et al. (10) for the determination of the total oestrogen content in amniotic fluid was evaluated This method gave unreliable results when the samples ere assayed ith the same technique as for rine The main technical difficulty was turbidity caused by precipitation of the proteins. However if the sample ... allowed to stand f 10 minutes after the hydrol

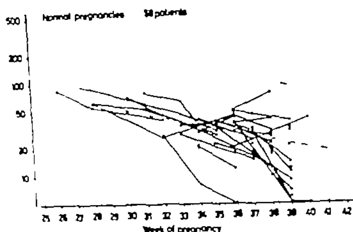


Fig. 1. CI values in normal pregnancies. The broken lines indicate the Liley zones.

carefully by a skilled technician the results were reproducible. Recovery experiments were made using a pool of amniotic fluid containing 0.160 μg oestrogen/ml. Determinations on the unfrozen pool ranged from 0.154 to 0.164 μg/ml (n = 4), and on the frozen pool from 0.158 to 0.176 μg/ml (n = 4). The addition of oestriol 0.200 μg/ml gave a recovery of 46%, 0.400 μg/ml 45%, 0.800 μg/ml 73% and 1.300 μg/ml 72% (mean of 4 determinations each). The coefficient of variation for 13 unfrozen samples with double determinations was 6.9%. The range for these samples was 0.077–0.970 μg/ml. These samples were kept in a refrigerator at +4 to 6°C for 30 to 45 days between the determinations. The storing of unfrozen samples for that length of time thus had little effect upon the oestrogen content. The coefficient of variation when comparing determinations of unfrozen and frozen samples was 7.2% (n = [illegible]).

On 172 consecutive samples from 110 pregnancies determinations of alkaline and acid phosphatases were performed according to the method described by Beckman (2) and Beckman et al. (3). 101 of these samples were from pregnancies with complete data on the mothers and infants and this series was subjected to stepwise regression analysis (13), program BMD 02 R (6). A detailed description of the phosphatase analyses and the results in normal pregnancies will be published elsewhere.

RESULTS

The following expressions will be used to denote statistical significance: Not significant ($P > 0.05$), probably significant ($0.05 > P > 0.01$), significant ($0.01 > P > 0.001$) and highly significant ($0.001 > P$).

CI Level

A. Normal pregnancies

The individual CI values showed a rather wide scatter which was especially pronounced during the last few weeks of pregnancy. The majority of values fell in the lowest Liley zone (Fig. 1). The borders of the Liley main zones are marked in the figures. In 23 patients repeated samples were taken and 5 of these showed increasing values.

The mean values followed rather closely the border line between the lower and the middle zones up to the 37th–38th week. From then on there was a more rapid decrease (Fig. 6, Table I).

B. Cases of Rh-isoimmunization

Group I. The individual values showed the same pattern as in the normal pregnancies. The CI values were mostly found in the lower zone or in the lower part of the middle zone (Fig. 2). Only two of 20 patients had an increase in values at repeated sampling.

The mean values were in the lower part of the middle zone and declined with advancing gestational age (Fig. 6). When the mean values were compared with the means from normal pregnancies a probably significant difference was found for the weeks 33–34 and 35–36. Later in pregnancy the differences were not significant (Table I).

Group II. The majority of the individual values fell in the lower part of the middle zone (Fig. 3). Increasing values were noted in 8 of 38 patients with repeated sampling.

The mean values were located centrally in the middle zone and showed the same sharp decline after the 37th–38th week as the normal pregnancies (Fig. 6). The mean values were only sig-

Table I *Mean ΔE_{450} values $\times 1000 \pm$ S.E.M*

Figures within brackets indicate number of samples. In Rh group IV cases were excluded which had complicating postnatal disease

Pregnancy week	Normal pregnancies	Diagnostic group Rh-isoim. group I	Rh-isoim. group II	Rh-isoim. group III	Rh-isoim. group IV	Hepatosis gravidarum
25–26	—	—	—	—	277 0 ±103 0 (3)	—
27–28	68.5 ±9 6 (4)	—	410 — (1)	250.8 ±31 1 (4)	229 8 ±46.1 (4)	—
29–30	68.0 — (2)	53.5 — (2)	227.5 — (2)	186.6 ±17 4 (13)	243.8 ±56.8 (5)	177.5 — (2)
31 32	43 7 ±6.5 (7)	120 — (1)	88.3 ±13.8 (9)	152.9 ±20.4 (15)	[illegible]34 1 ±27 0 (8)	295.5 — (2)
33–34	33 0 ±4 4 (11)	64 0 ±14.2 (6)	68.6 ±6.6 (23)	124.8 ±12.5 (28)	318 4 ±64 1 (9)	160 — (1)
35–36	30.9 ±2.5 (22)	47 6 ±6.7 (17)	52.8 ±13.8 (38)	104 0 ±14 9 (35)	170.3 ±76.3 (4)	125.3 ±45.4 (4)
37–38	23 6 ±2.6 (28)	28.0 ±2.8 (40)	39.5 ±3 1 (55)	8[illegible].8 ±13 0 (24)	220.0 ±49 4 (3)	99 9 ±26.1 (6)
39–40	14 1 ±2.5 (23)	23 2 ±4.3 (31)	14.5 ±5 1 (4)	45 — (1)	—	30.8 ±7 8 (6)

nificantly different from the group I means for the 37th–38th week (Table I).

Group III The individual CI values usually fell in the upper part of the middle zone or in the upper zone. 15 of 35 patients with repeated sampling showed an increase in values (Fig. 4).

The mean values were found in the upper part of the middle zone and showed a decline parallel

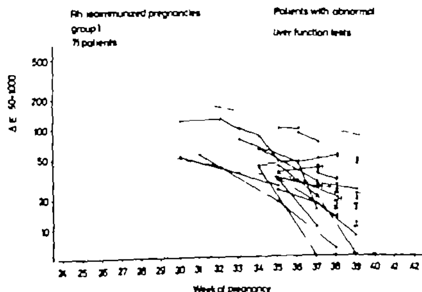


Fig. 2 Individual CI values for cases with slight haemolytic disease.

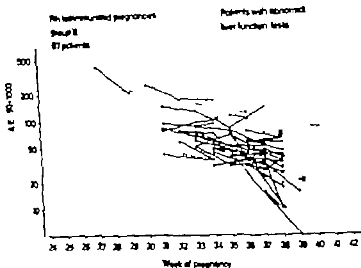


Fig. 3. Individual CI values for cases with moderate haemolytic disease.

to the slope of the Liley lines (Fig. 6). The difference between the means *for* Rh *groups II and* III was probably significant for the 31st–32nd week and highly significant for the later periods (Table I).

Group IV. Most of the individual values fell in the upper zone and 5 of 11 cases with repeated sampling showed an increase in values (Fig. 5). If those cases were excluded where erythroblastosis was not the main cause of fetal death all values fell within the upper zone.

The mean values did not show the usual decline with advancing pregnancy but instead a rather constant level (Fig. 6). The mean values were calculated only with the determinations from pure cases of erythroblastosis. The difference between *groups III and IV was probably significant* for the 31st–32nd week and highly significant for the following periods (Table I).

C. *Hepatosis gravidarum*

The CI values usually fell within the middle or upper zone (Fig. 7). The mean values were not significantly different from the Rh group III mean values for comparable periods (Table I).

D. *Patients with other complications of pregnancy*

Three of four samples from patients with polyhydramnios had low CI values compared with

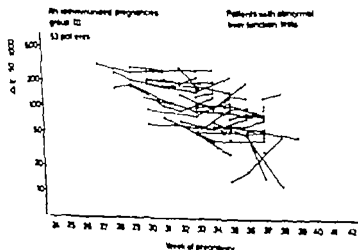


Fig. 4. Individual CI values for cases with severe haemolytic disease and surviving infants.

Table I *Mean* ΔE_{450} *values* $\times$ *1000* $\pm$ *S.E.M.*

Figures within brackets indicate number of samples. In Rh group IV cases were excluded which had complicating [illegible] disease

Pregnancy week	Normal pregnancies	Diagnostic group: Rh-isoim. group I	Rh-isoim. group II	Rh-isoim. group III	Rh-isoim. group IV	Hepatosis gravidarum
25–26	—	—	—	—	277.0 ±103.0 (3)	—
27–28	68.5 ±9.6 (4)	—	410 — (1)	[illegible]30.8 ±31.1 (4)	[illegible]29.8 ±46.1 (4)	—
29–30	68.0 — (2)	53.5 — (2)	227.5 — (2)	186.6 ±17.4 (13)	43.8 ±56.8 (5)	177.5 — ([illegible])
31–32	43.7 ±6.5 (7)	120 — (1)	88.3 ±13.8 (9)	15[illegible].9 ±20.4 (15)	[illegible]34.1 ±27.0 (8)	295.5 — (2)
33–34	33.0 ±4.4 (11)	64.0 ±14.2 (6)	68.6 ±6.6 (23)	1[illegible]4.8 ±12.5 (28)	318.4 ±64.1 (9)	160 — (1)
35–36	30.9 ±[illegible].5 (22)	47.6 ±6.7 (17)	5[illegible].8 ±13.8 (38)	104.0 ±14.9 (35)	170.3 ±[illegible]6.3 (4)	125.3 ±45.4 (4)
37–38	[illegible]3.6 ±[illegible].6 ([illegible]8)	28.0 ±2.8 (50)	39.5 ±3.1 (55)	82.8 ±13.0 ([illegible]4)	220.0 ±49.4 (3)	99.9 ±[illegible]6.1 (6)
39–40	14.1 ±2.5 (23)	23.2 ±4.3 (31)	14.5 ±5.1 (4)	45 — (1)	—	30.8 ±7.8 (6)

nificantly different from the group I means for the 37th–38th week (Table I).

Group III The individual CI values usually fell in the upper part of the middle zone or in the upper zone 15 of 35 patients with repeated sampling showed an increase in values (Fig. 4).

The mean values were found in the upper part of the middle zone and showed a decline parallel

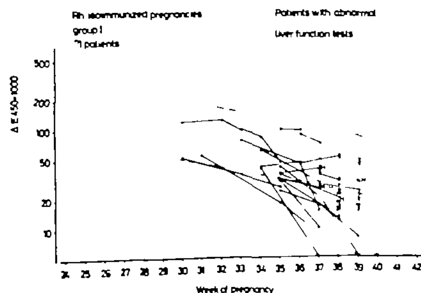


Fig. [illegible] Individual CI values for cases with slight haemolytic disease.

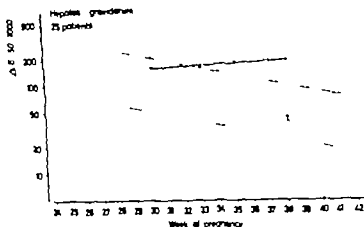


Fig. 7. Individual CI values for nonimmunized cases with hepatosis gravidarum. Samples with visible meconium contamination excluded.

correlation was highly significant in the 37th–38th week (=50 r=0.45), samples before the 37th week were too few for calculation.

CI and maternal serum bilirubin

Normal pregnancies showed no significant correlation between the CI values and the maternal serum bilirubin concentrations (=53). However this correlation was highly significant for nonimmunized patients with hepatosis gravidarum (r=0.81 =76)

T elve patients in Rh groups I–IV had abnormal liver function tests with the same pattern as in patients with hepatosis gravidarum. Some of them had CI alues high in the range for their Rh groups. Those high values all belonged to patients with elevated maternal serum bilirubin.

CI and amniotic fluid total protein level

The results of the protein estimations were published previously (23). Normal pregnancies showed a highly significant correlation between the CI alues and the total protein concentrations in the 26th–36th week (r=0.87 n=79). In the 37th–40th eek the correlation was significant (r=0.53 30)

Rh-nonimmunized patients groups III and IV also showed highly significant correlation between CI and total protein levels in the 35th–36th eek (r=0.64 =90). In the 37th–40th eek the correlation was significant (r=0,70 = 16). For pregnancies groups I II the correlation was also highly significant in the 31st 36th week (=0.60 n=69) and significant in the 37th–40th eek (r=0.33 =81).

Nonimmunized patients with hepatosis gravidarum showed no significant correlation between CI values and total protein levels.

Ratio CI/Amniotic fluid total protein levels

The quotients between the CI values and the total protein concentrations were calculated for the clinical groups (Table II). For normal pregnancies and cases of Rh-isoimmunization group I–III the mean quotients were rather constant up to the 35th–36th week and then a sharp decline occurred during the last few weeks of pregnancy. The few values in group IV indicated a rising tendency from the 31st–32nd week. The differences between the normal pregnancies and Rh group I was significant only for the 35th–36th week. The differences between Rh groups I and II were probably significant in the weeks 35–36 and significant in the weeks 37–38. A probably significant difference between groups II and III occurred in the 31st–32nd week and highly significant differences in the periods up to the 37th–38th week. The difference between groups III and IV was significant only in the 37th–38th week. No significant correlation was found between the ratio and infant cord haemoglobin values in Rh groups I IV for infants delivered within 7 days of sampling (n=6). A highly significant correlation was found with cord serum bilirubin levels for the same categories (=0.63 n=83)

CI and infant birth weight

No significant correlation could be found between the CI values and infant birth weight in

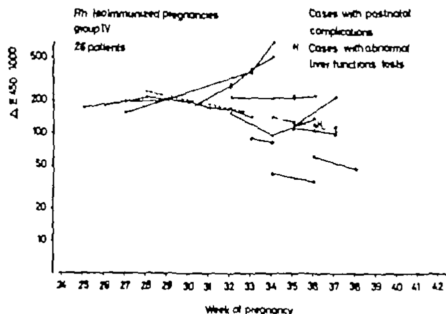


Fig. 5. Individual CI values for cases with ultimate perinatal fetal death.

normal pregnancies of the same duration. The fourth sample showed a very high value and this infant had an oesophageal atresia. Another high value occurred in a case with intestinal atresia without polyhydramnios. Two cases with diabetes and two with preeclampsia had values within the normal range.

CI and cord blood haemoglobin

No significant correlation existed between CI values and cord blood haemoglobin concentrations for normal infants delivered within 7 days of sampling ($n = 29$). For infants belonging to Rh groups III and IV the correlation between CI and cord blood haemoglobin was highly significant both in the 35th–36th and in the 37th–38th week ($n = 14$ and 22, $r = -0.65$ and -0.73). For Rh groups I–II the correlation was not significant in the weeks 37–38 ($n = 51$), samples before the 37th week were too few for calculation.

CI and cord serum bilirubin

In normal pregnancies no significant correlation was found between CI values and cord serum bilirubin concentrations for infants delivered within 7 days of sampling ($n = 28$).

For Rh isoimmunized patients group III–IV the corresponding correlation was not significant in the 35th–36th week nor in the 37th–38th week ($n = 18$ and 23). For pregnancies group I–II the

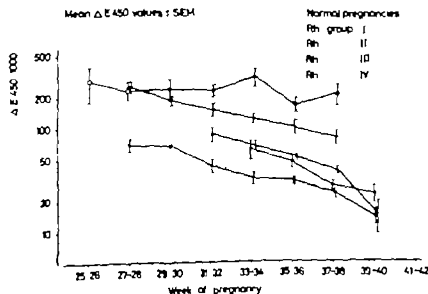


Fig. 6. Mean CI values for normal pregnancies and for pregnancies complicated by haemolytic disease. Cases with postnatal complications are excluded from group IV.

Table III *Acid phosphatase in amniotic fluid*

Activity expressed as μmol p-nitrophenol liberated per ml and hour. Figures within brackets indicate number of samples. Means ± S.E.M.

Pregnancy week	Normal pregnancies	Rh group I	Rh group II	Rh group III	Rh group IV
27–28	0.10 — (1)	—	—	0.18 (2)	—
29–30	0.11 (1)	—	—	0.31 ±0.11 (5)	—
31–32	—	—	0.16 ±0.05 (4)	0.17 ±0.04 (5)	0.19 (2)
33–34	0.11 ±0.04 (3)	0.11 (2)	0.16 ±0.05 (10)	0.15 ±0.02 (8)	0.12 (2)
35–36	0.16 ±0.03 (11)	0.21 ±0.06 (8)	0.21 ±0.05 (12)	0.20 ±0.03 (10)	0.13 (2)
37–38	0.13 ±0.02 (15)	0.26 ±0.07 (11)	0.26 ±0.06 (10)	0.23 ±0.05 (6)	—
39–40	0.20 0.03 (17)	0.14 — (1)	0.18 ±0.04 (3)	—	—
41–42	0.31 0.08 (4)	—	—	—	—

43rd week period ($r = 0.95$, $n = 62$). For the Rh-immunization group I–IV the correlation between the progesterone levels and the CI values was highly significant during the same period (= 0.42, = 166) as was also the correlation with total protein levels ($r = 0.53$, $n = 164$). Rh-immunized patients group I–IV delivered within 7 days of sampling showed probably significant correlation between the progesterone concentrations and cord blood haemoglobin levels ($r = 0.34$, $n = 45$) but no significant correlation with cord serum bilirubin values (= 50).

Total oestrogen in amniotic fluid

The oestrogen determinations in most cases gave unreliable results for technical reasons. From 33 samples, however, repeated determinations gav reproducible results. The low number of samples in the various clinical groups prevented statistical analysis. T o normal pregnancies at 30–3. weeks yielded values of 0.055 and 0.043 μg oestrogen/ml amniotic fluid. Six samples from pregnancies belonging to Rh groups I and II at 37–40 weeks had values with the range 0.148–0.989 μg/ml. One pregnancy group III showed an increase from 0.100 μg/ml t 28 weeks to 0.288 μg/ml at 34 weeks. One pregnancy group II increased from 0.079 t 31 weeks to 0.148 μg/ml at 37 weeks. One pregnancy group IV had 0.035 μg/ml at 26 weeks and 0.048 μg/ml at 33 weeks. Three other group IV pregnancies had 0.042 at 31 weeks, 0.079 at 33 weeks and 0.058 μg/ml at 35 weeks respectively.

Acid phosphatases in amniotic fluid

The acid phosphatase activity showed slow increase with advancing pregnancy both in normal cases and in cases with haemolytic disease (Table III). The increase was not significant for the periods observed, nor were the differences significant between the groups. A few samples from patients with hydramnios or preeclampsia gave values within the range for normal pregnancies. Patients with hepatosis had a tendency to elevated values.

Table II *The quotient between ΔCI_{450} values and amniotic fluid total protein content*

Means ± S.E.M. Figures within brackets indicate number of samples. Cases were excluded which had post-natal complications

Pregnancy week	Normal pregnancies	Diagnosis: Rh group I	Rh group II	Rh group III	Rh group IV
25–26	0.157 — (1)	—	—	—	0.409 ±0.051 (3)
27–28	0.121 — (1)	—	—	0.414 ±0.029 (4)	0.364 (2)
29–30	0.170 — (2)	—	—	0.335 ±0.032 (8)	0.374 ±0.022 (4)
31–32	0.123 — (2)	0.145 — (1)	0.195 ±0.025 (5)	0.313 ±0.053 (11)	0.427 ±0.046 (6)
33–34	0.09? ±0.011 (7)	0.145 ±0.037 (3)	0.194 ±0.015 (?0)	0.351 ±0.032 (18)	0.481 ±0.087 (4)
3?–36	0.108 ±0.010 (16)	0.147 ±0.014 (14)	0.197 ±0.018 (26)	0.396 ±0.068 (22)	0.530 ±0.116 (3)
37–38	0.099 ±0.011 (17)	0.103 ±0.011 (27)	0.158 ±0.015 (34)	0.290 ±0.037 (14)	0.618 ±0.054 (3)
39–40	0.066 ±0.013 (13)	0.089 ±0.018 (18)	0.057 ±0.030 (3)	—	—

28 normal pregnancies with delivery within 7 days of sampling. All these infants weighed more than 2 500 g. CI values of 10 or lower occurred in 10 of these cases, with three infants weighing between 2 500 and 3 000 g. Values of ?0 or lower occurred in 18 cases with 5 infants weighing 2 500 to 3 000 g.

Taking normal pregnancies and Rh group I together 73 infants were delivered within 7 days of sampling. CI values of 10 or lower occurred in 23 of these cases. All these infants weighed more than 2 500 g, four weighed 2 500 to 3 000 g. CI values of ?0 or lower were encountered in 46 cases, one infant weighed under 2 500 g, nine 2 500 to 3 000 g. In Rh groups I and II ten infants weighed under ? 500 g. The lowest CI value for any of these was 16.

Cord blood haemoglobin, cord serum bilirubin and exchange transfusions

Rh groups I–IV showed a highly significant correlation between cord blood haemoglobin and cord serum bilirubin concentrations ($r = -0.59$ $n = 203$). For Rh groups I–III a highly significant correlation existed between the cord blood haemoglobin concentrations and the number of exchange transfusions given to the babies ($r = -0.61$ $n = 181$). The correlation between cord serum bilirubin concentrations and the number of exchange transfusions was also highly significant ($r = 0.72$, $n = 194$)

Progesterone in amniotic fluid compared with CI amniotic fluid total protein cord blood haemoglobin and cord serum bilirubin

Results of the progesterone determinations for the various clinical groups were published separately (?1).

A highly significant correlation between progesterone levels in amniotic fluid and the CI values ($r = 0.50$ $n = 44$) existed for normal pregnancies in the 26th–39th week period. The progesterone levels also showed a highly significant correlation to the total protein concentrations in the 26th–

Table III. *Acid phosphatase in amniotic fluid*

Activity expressed as μmol α-naphthol liberated per ml and hour. Figures within brackets indicate number of samples. Means ± S.E.M.

Pregnancy week	Normal pregnancies	Rh group I	Rh group II	Rh group III	Rh group IV
27–28	0.10 — (1)	—	—	0.18 (2)	—
29–30	0.11 (1)	—	—	0.31 ±0.11 (5)	—
31–32	—	—	0.16 ±0.09 (4)	0.17 ±0.04 (5)	0.19 (2)
33–34	0.11 ±0.04 (3)	0.11 (2)	0.18 ±0.05 (10)	0.15 ±0.03 (8)	0.12 (2)
35–36	0.16 ±0.03 (11)	0.21 ±0.06 (8)	0.21 ±0.05 (12)	0.20 ±0.03 (10)	0.13 (2)
37–38	0.15 ±0.02 (13)	0.26 ±0.07 (11)	0.26 ±0.06 (10)	0.25 ±0.05 (6)	—
39–40	0.29 ±0.03 (17)	0.14 — (1)	0.18 ±0.04 (3)	—	—
41–42	0.31 ±0.08 (4)	—	—	—	—

43rd week period ($r = 0.95$, $n = 6$). For the Rh-immunization group I–IV the correlation between the progesterone levels and the CI values was highly significant during the same period ($r = 0.42$, $n = 166$) as was also the correlation with total protein levels ($r = 0.53$, $n = 164$). Rh-immunized patients group I–IV delivered within 7 days of sampling showed a probably significant correlation bet[w]een the progesterone concentrations and cord blood haemoglobin levels ($r = 0.34$, $n = 45$) but no significant correlation with cord serum bilirubin [va]lues ($n = 50$).

Total oestrogen in amniotic fluid

The oestrogen determinations in most cases gav[e] unreliable results for technical reasons. From 33 samples, however, repeated determinations ga[v]e reproducible results. The low number of samples in the various clinical groups prevented statistical analysis. Two normal pregnancies at 30–32 weeks yielded values of 0.053 [a]nd 0.043 μg oestrogen/ml amniotic fluid. Six samples from pregnancies belonging to Rh groups I and II at 37–40 weeks had values with the range 0.148–0.989 μg/ml. One pregnancy group III showed an increase from 0.100 μg/ml at 28 weeks to 0.288 μg/ml at 34 weeks. One pregnancy group II increased from 0.079 at 31 weeks to 0.148 μg/ml at 37 weeks. One pregnancy group IV had 0.035 μg/ml at 26 weeks and 0.048 μg/ml at 33 weeks. Three other group IV pregnancies had 0.042 at 31 weeks, 0.079 at 33 weeks and 0.058 μg/ml at 35 weeks respectively.

Acid phosphatases in amniotic fluid

The acid phosphatase activity showed a slow increase with advancing pregnancy both in normal cases and in cases with haemolytic disease (Table III). The increase was not significant for the periods observed, nor were the differences significant between the groups. A few samples from patients with hydramnios or preeclampsia gave values within the range for normal pregnancies. Patients with hepatosis had a tendency to elevated values.

Table II *The quotient between* ΔCI_{450} *values and amniotic fluid total protein content*

Means ± S.E.M Figures within brackets indicate number of samples. Cases were excluded which had post-natal complications

Pregnancy week	Normal pregnancies	Diagnosis			
		Rh group I	Rh group II	Rh group III	Rh group IV
25–26	0.157 — (1)	—	—	—	0.409 ±0.051 (3)
27–28	0 121 — (1)	—	—	0.414 ±0.029 (4)	0.364 (2)
29–30	0.170 — (2)	—	—	0.335 ±0.032 (8)	0.374 ±0.022 (4)
31 32	0.123 — (2)	0.145 — (1)	0.195 ±0.025 (5)	0.313 ±0.053 (11)	0.427 ±0.046 (6)
33–34	0.092 ±0.011 (7)	0.145 ±0 037 (3)	0.194 ±0.015 (20)	0.351 ±0.032 (18)	0 481 ±0 087 (4)
3 36	0.108 ±0.010 (16)	0.147 ±0.014 (14)	0.197 ±0.018 (26)	0.396 ±0.068 (22)	0.530 ±0.116 (3)
37–38	0.099 ±0.011 (17)	0.103 ±0 011 (27)	0.158 ±0.015 (34)	0.290 ±0.037 (14)	0.618 ±0.054 (3)
39–40	0.066 ±0.013 (13)	0.089 ±0.018 (18)	0.057 ±0.030 (3)	—	—

28 normal pregnancies with delivery within 7 days of sampling. All these infants weighed more than 2 500 g. CI values of 10 or lower occurred in 10 of these cases, with three infants weighing between 2 500 and 3 000 g Values of 20 or lower occurred in 18 cases with 5 infants weighing 2 500 to 3 000 g.

Taking normal pregnancies and Rh group I together 73 infants were delivered within 7 days of sampling. CI values of 10 or lower occurred in 23 of these cases. All these infants weighed more than 2 500 g. four weighed 2 500 to 3 000 g. CI values of 20 or lower were encountered in 46 cases, one infant weighed under 2 500 g. nine 2 500 to 3 000 g. In Rh groups I and II ten infants weighed under 2 500 g. The lowest CI value for any of these was 16.

Cord blood haemoglobin cord serum bilirubin and exchange transfusions

Rh groups I–IV showed a highly significant correlation between cord blood haemoglobin and cord serum bilirubin concentrations ($r = -0.59$ $n = 203$). For Rh groups I–III a highly significant correlation existed between the cord blood haemoglobin concentrations and the number of exchange transfusions given to the babies ($r = -0.61$ $n = 181$). The correlation between cord serum bilirubin concentrations and the number of exchange transfusions was also highly significant ($r = 0.72$, $n = 194$)

Progesterone in amniotic fluid compared with CI amniotic fluid total protein cord blood haemoglobin and cord serum bilirubin

Results of the progesterone determinations for the various clinical groups were published separately (21).

A highly significant correlation between progesterone levels in amniotic fluid and the CI values ($r = 0.50$ $n = 44$) existed for normal pregnancies in the 26th–39th week period. The progesterone levels also showed a highly significant correlation to the total protein concentrations in the 6th–

Table III. *Acid phosphatase in amniotic fluid*

Activity expressed as μmol α-naphtol liberated per ml and hour. Figures within brackets indicate number of samples. Means ± S.E.M.

Pregnancy week	Normal pregnancies	Rh group I	Rh group II	Rh group III	Rh group IV
27–28	0.10 — (1)	—	—	0.18 (2)	—
29–30	0.11 (1)	—	—	0.31 ±0.11 (5)	—
31–32	—	—	0.16 ±0.09 (4)	0.17 ±0.04 (5)	0.19 (2)
33–34	0.11 ±0.04 (3)	0.11 (2)	0.16 ±0.05 (10)	0.15 ±0.02 (8)	0.12 (2)
35–36	0.16 ±0.03 (11)	0.21 ±0.06 (8)	0.21 ±0.05 (12)	0.20 ±0.03 (10)	0.13 (2)
37–38	0.15 ±0.02 (13)	0.26 ±0.07 (11)	0.26 ±0.06 (16)	0.25 ±0.05 (6)	—
39–40	0.20 ±0.03 (17)	0.14 — (1)	0.18 ±0.04 (3)	—	—
41–42	0.31 ±0.08 (4)	—	—	—	—

43rd week period ($r = 0.95$, $n = 62$). For the Rh-immunization group I–IV the correlation between the progesterone levels and the OD values was highly significant during the same period ($r = 0.42$, $n = 166$) as was also the correlation with total protein levels ($r = 0.53$, $n = 164$). Rh-immunized patients group I–IV delivered within 7 days of sampling showed a probably significant correlation between the progesterone concentrations and cord blood haemoglobin levels ($r = 0.34$, $n = 45$) but no significant correlation with cord serum bilirubin values ($n = 50$).

Total oestrogen in amniotic fluid

The oestrogen determinations in most cases gave unreliable results for technical reasons. From 33 samples, however, repeated determinations gave reproducible results. The low number of samples in the various clinical groups prevented statistical analysis. Two normal pregnancies at 30–32 weeks yielded values of 0.055 and 0.043 μg oestrogen/ml amniotic fluid. Six samples from pregnancies belonging to Rh groups I and II at 37–40 weeks had values with the range 0.148–0.989 μg/ml. One pregnancy group III showed an increase from 0.100 μg/ml at 28 weeks to 0.288 μg/ml at 34 weeks. One pregnancy group II increased from 0.079 at 31 weeks to 0.148 μg/ml at 37 weeks. One pregnancy group IV had 0.035 μg/ml at 26 weeks and 0.048 μg/ml at 33 weeks. Three other group IV pregnancies had 0.042 at 31 weeks, 0.079 at 33 weeks and 0.058 μg/ml at 35 weeks respectively.

Acid phosphatases in amniotic fluid

The acid phosphatase activity showed a slow increase with advancing pregnancy both in normal cases and in cases with haemolytic disease (Table III). The increase was not significant for the periods observed, nor were the differences significant between the groups. A few samples from patients with hydramnios or preeclampsia gave values within the range for normal pregnancies. Patients with hepatosis had a tendency to elevated values.

Table II. *The quotient between ΔCI_{414} values and amniotic fluid total protein content*

Means ± S.E.M. Figures within brackets indicate number of samples. Cases were excluded which had post-natal complications

Pregnancy week	Normal pregnancies	Diagnosis: Rh group I	Rh group II	Rh group III	Rh group IV
25–26	0.157 — (1)	—	—	—	0.409 ±0.051 (3)
27–28	0.121 — (1)	—	—	0.414 ±0.029 (4)	0.364 (2)
29–30	0.170 — (2)	—	—	0.335 ±0.032 (8)	0.374 ±0.022 (4)
31–32	0.123 — (7)	0.145 — (1)	0.195 ±0.025 (5)	0.313 ±0.053 (11)	0.427 ±0.046 (6)
33–34	0.092 ±0.011 (7)	0.145 ±0.037 (3)	0.194 ±0.015 (20)	0.351 ±0.032 (18)	0.481 ±0.087 (4)
35–36	0.108 ±0.010 (16)	0.147 ±0.014 (14)	0.197 ±0.018 (26)	0.396 ±0.068 (22)	0.530 ±0.116 (3)
37–38	0.099 ±0.011 (17)	0.103 ±0.011 (27)	0.158 ±0.015 (34)	0.290 ±0.037 (14)	0.618 ±0.054 (3)
39–40	0.066 ±0.013 (13)	0.089 ±0.018 (18)	0.057 ±0.030 (3)	—	—

28 normal pregnancies with delivery within 7 days of sampling. All these infants weighed more than 2 500 g. CI values of 10 or lower occurred in 10 of these cases, with three infants weighing between 2 500 and 3 000 g. Values of 20 or lower occurred in 18 cases with 5 infants weighing 2 500 to 3 000 g.

Taking normal pregnancies and Rh group I together 73 infants were delivered within 7 days of sampling. CI values of 10 or lower occurred in 23 of these cases. All these infants weighed more than 2 500 g, four weighed 2 500 to 3 000 g. CI values of 20 or lower were encountered in 46 cases, one infant weighed under 2 500 g, nine 2 500 to 3 000 g. In Rh groups I and II ten infants weighed under 2 500 g. The lowest CI value for any of these was 16.

Cord blood haemoglobin, cord serum bilirubin and exchange transfusions

Rh groups I–IV showed a highly significant correlation between cord blood haemoglobin and cord serum bilirubin concentrations ($r = -0.59$, $n = 203$). For Rh groups I–III a highly significant correlation existed between the cord blood haemoglobin concentrations and the number of exchange transfusions given to the babies ($r = -0.61$, $n = 181$). The correlation between cord serum bilirubin concentrations and the number of exchange transfusions was also highly significant ($r = 0.72$, $n = 194$).

Progesterone in amniotic fluid compared with CI, amniotic fluid total protein, cord blood haemoglobin and cord serum bilirubin

Results of the progesterone determinations for the various clinical groups were published separately (21).

A highly significant correlation between progesterone levels in amniotic fluid and the CI values ($r = 0.50$, $n = 44$) existed for normal pregnancies in the 6th–39th week period. The progesterone levels also showed a highly significant correlation to the total protein concentrations in the 26th–

values and total protein concentrations. Probably significant relationships were found with the week of pregnancy to blood factor 0 of the infant and Rh factor of mother (Fig. 10).

The amniotic fluid protein content had highly significant relationships with the week of pregnancy and CI values. Significant influences were exercised by order of pregnancy and cord serum bilirubin alues. A probably significant relationship occurred with blood factor 0 of the mother (Fig. 11).

Alkaline phosphatase activity had a highly significant connection with the CI values and a probably significant connection with the week of pregnancy (Fig. 12).

The acid phosphatase activity was not found to be significantly influenced by any of the stu died variables. The regression coefficients and their F-values are given in the figures for the variables found to have significant effects.

Amniotic fluid total prote and the order f pregnancy

To test whether pregnancy order might influence the amniotic fluid protein concentrations the total number of late normal pregnancies was analysed. Linear regression analysis of the values from gravidae I II in the eeks 27–43 gave the equation $y = 1032.07 - 20.85$ $(n = 36)$. The corresponding equation for gravida $\geq$ III was $y = 654.56 - 10.69$ $x(n = 36)$ y then mg protein/100 ml and x the duration of pregnancy in weeks. The slope of the regression lines wa significantly different. This indicated that the decrease of the protein concentration ith advancing pregnancy was slower for the patients with higher order of pregnancy. The regression lines cut each other in the 37th eek.

DISCUSSION

No general agreement exists concerning the classification of pregnancies complicated by Rh-immunization. The model chosen in this study also takes into account the number of exchange transfusions to the infants. The same principle has been used by others even if the criteria of classification has been somewhat different (8, 11 27 73, 40, 41). The limit between cases with slight and moderate haemolytic disease (Rh groups I and II) was fixed t a haemoglobin value

Table V. *Variables used in multiple stepwise regression analysis*

Code	Variable	Mean ± S.D.
ID	Clinical isoimmunization group	– –
PW	Week of pregnancy	35.70 ± 2.40
PO	Order of pregnancy	3.06 ± 1.22
S	Sex of infant (male +1 female −1)	−0.09 –
AI	Blood factor A infant (present +1 absent −1)	0.05 –
BI	Blood factor B infant (present +1 absent −1)	−0.66 –
OI	Blood factor 0 infant (present +1 absent −1)	−0.31 –
RhI	Rh factor of infant (positive +1 negative −1)	0.55 –
MA	Age of mother	28.04 ± 5.29
AM	Blood factor A of mother (present +1 absent −1)	0.23 –
BM	Blood factor B of mother (present +1 absent −1)	−0.60 –
OM	Blood factor 0 of mother (present +1 absent −1)	−0.52 –
RhM	Rh factor of mother (positive +1 negative −1)	−0.79 –
CI	Amniotic fluid CI	58.01 ± 40.08
Hbl	Cord blood haemoglobin, g/100 ml	13.21 ± 4.26
Bil	Cord serum bilirubin, mg/100 ml	3.26 ± 1.90
TP	Amniotic fluid total protein mg/100 ml	291.27 ± 107.34
Ac	Acid phosphatase activity μmol/ml, h	0.20 ± 0.15
Alk	Alkaline phosphatase activity μmol/ml h	1.48 ± 2.37

of 12.1/100 ml because in the present series o normal pregnancies the mean cord blood haemo globin value was 15.7 ± 3.6 (2 S.D.) g/100 m $(n = 55)$. The haemoglobin value 8.1 g/100 m was selected as the lower limit for cases wit moderate disease as all cases with perinatal deat due to erythroblastosis alone had cord bloo haemoglobin values below this limit.

It was not the aim of the present study t evaluate the clinical results of our managemen of the Rh-isoimmunized patients. However it ma be pointed out that the overall perinatal mortalit rate in this series was 12.7% (30 of 237 cases

Table IV *Alkaline phosphatase in amniotic fluid*

Activity expressed as μmol α-naphtol liberated per ml and hour Means ± S.E.M Figures within brackets indicate number of samples

Pregnancy week	Normal pregnancies	Rh group I	Rh group II	Rh group III	Rh group IV
27 28	0.53 — (1)	—	—	1.96 — (2)	—
29–30	0.63 — (1)	—	—	1.21 ±0.40 (5)	—
31 32	—	—	0.70 ±0.23 (4)	0.91 ±0.39 (5)	0.25 — (1)
33–34	0.78 ±0.22 (3)	0 75 — (2)	0.84 ±0.12 (10)	0 75 ±0.13 (8)	0.32 — (2)
35–36	0.93 ±0.17 (11)	1 16 ±0.21 (8)	1.26 ±0.21 (12)	0.86 ±0.15 (10)	0.67 — (2)
37–38	1 66 ±0.30 (13)	1 04 ±0.19 (10)	1.87 ±0.36 (10)	1.35 ±0.27 (6)	—
39–40	[illegible].98 ±0.69 (17)	0.84 — (1)	2.04 ±0 63 (3)	—	—
41–42	3 66 ±0.51 (4)	—	—	—	—

Staining with meconium did not seem to affect the levels significantly

Alkaline phosphatases in amniotic fluid

The alkaline phosphatase activity showed an increase with advancing pregnancy and for normal pregnancies the increase was probably significant from the 35th–36th week to the 39th–40th week (Table IV) Also for Rh group II the rise was probably significant from the 33rd–34th to the 37th–38th week. The few values in Rh group IV fell into the lower range of the other groups. The differences for the other groups were not significant. Nonimmunized patients with hepatosis gravidarum had a tendency to low values compared with the normals. Samples from patients with hydramnios or preeclampsia gave values within the normal range. Samples contaminated by visible amounts of meconium showed a very high activity exceeding normal levels 20–400 times.

In normal pregnancies a probably significant correlation was found between the alkaline phosphatase activities in amniotic fluid and maternal serum ($r = 0\ 37$ $n = 30$).

Multiple regression analysis

The analysis was performed with the variables listed in Table V When CI was the dependent variable highly significant relationships were found with the total protein levels in amniotic fluid, with the alkaline phosphatase activity in amniotic fluid significant relationships with the blood factor 0 of the mother and with the cord serum bilirubin concentration. A probably significant relationship existed with the blood factor A of the mother (Fig. 8)

Cord blood haemoglobin values were probably significantly influenced by the clinical classification group and by blood factor 0 of the infant (Fig. 9).

Cord serum bilirubin concentrations were influenced to a highly significant degree by the clinical group and the Rh factor of the infant. Significant relationships were found with CI

values and total protein concentrations. Probably significant relationships were found with the week of pregnancy to blood factor 0 of the infant and Rh factor of mother (Fig. 10).

The amniotic fluid protein content had highly significant relationships with the week of pregnancy and CI values. Significant influences were exercised by order of pregnancy and cord serum bilirubin values. A probably significant relationship occurred with blood factor 0 of the mother (Fig. 11).

Alkaline phosphatase activity had a highly significant connection with the CI values and a probably significant connection with the week of pregnancy (Fig. 12).

The acid phosphatase activity was not found to be significantly influenced by any of the studied variables. The regression coefficients and their F-values are given in the figures for the variables found to have significant effects.

Amniotic fluid total protein and the order of pregnancy

To test whether pregnancy order might influence the amniotic fluid protein concentrations the total number of late normal pregnancies was analysed. Linear regression analysis of the values from gravidae I II in the weeks 27–43 gave the equation $y = 1031.07 - 20.85$ ($n = 36$). The corresponding equation for gravidae ≥ III was $y = 654.56 - 10.69$ x ($n = 36$); y was then mg protein/100 ml and x the duration of pregnancy in weeks. The slope of the regression lines was significantly different. This indicated that the decrease of the protein concentration with advancing pregnancy was slower for the patients with higher order of pregnancy. The regression lines cut each other in the 37th week.

Table V *Variables used in multiple stepwise regression analysis*

Code	Variable	Mean ± S.D
ID	Clinical Isoimmunization group	– –
PW	Week of pregnancy	35.70 ± 2.40
PO	Order of pregnancy	3.06 ± 1.22
S	Sex of infant (male +1 female −1)	−0.09 –
AI	Blood factor A infant (present +1 absent −1)	0.05 –
BI	Blood factor B infant (present +1 absent −1)	−0.66 –
OI	Blood factor 0 infant (present +1 absent −1)	−0.31 –
RhI	Rh factor of infant (positive +1 negative −1)	0.65 –
MA	Age of mother	28.04 ± 5.29
AM	Blood factor A of mother (present +1 absent −1)	0.23 –
BM	Blood factor B of mother (present +1 absent −1)	−0.60 –
OM	Blood factor 0 of mother (present +1 absent −1)	−0.52 –
RhM	Rh factor of mother (positive +1 negative −1)	−0.79 –
CI	Amniotic fluid CI	58.01 ± 60.08
HbI	Cord blood haemoglobin, g/100 ml	13.21 ± 4.26
Bil	Cord serum bilirubin, mg/100 ml	3.26 ± 1.90
TP	Amniotic fluid total protein mg/100 ml	291.27 ± 107.34
Ac	Acid phosphatase activity μmol/ml/h	0.20 ± 0.15
Alk	Alkaline phosphatase activity μmol/ml/h	1.48 ± 2.37

DISCUSSION

No general agreement exists concerning the classification of pregnancies complicated by isoimmunization. The model chosen in this study also takes into account the number of exchange transfusions to the infants. The same principle has been used by others even if the criteria of classification have been somewhat different (8, 11, 27, 38, 40, 41). The limit between cases with slight and moderate haemolytic disease (Rh groups I and II) was fixed at a haemoglobin value of 12.1/100 ml because in the present series of normal pregnancies the mean cord blood haemoglobin value was 15.7 ± 3.6 (2 S.D.) g/100 ml ($n = 55$). The haemoglobin value 8.1 g/100 ml was selected as the lower limit for cases with moderate disease as all cases with perinatal death due to erythroblastosis alone had cord blood haemoglobin values below this limit.

It was not the aim of the present study to evaluate the clinical results of our management of the Rh-isoimmunized patients. However it may be pointed out that the overall perinatal mortality rate in this series was 12.7 % (30 of 237 cases).

Table IV *Alkaline phosphatase in amniotic fluid*

Activity expressed as μmol α-naphtol liberated per ml and hour Means ± S.E.M. Figures within brackets indicate number of samples

Pregnancy week	Normal pregnancies	Rh group I	Rh group II	Rh group III	Rh group IV
27–28	0.53 — (1)	—	—	1 96 — (2)	—
29–30	0 63 — (1)	—	—	1.21 ±0.40 (5)	—
31 32	—	—	0.70 ±0.[illegible]3 (4)	0.91 ±0.39 (5)	0.[illegible]5 — (1)
33–34	0.78 ±0.2[illegible] (3)	0.75 — (2)	0.84 ±0.1 (10)	0.75 ±0.13 (8)	0.3[illegible] — (2)
35–36	0.93 ±0.17 (11)	1 16 ±0.21 (8)	1.26 ±0.21 (12)	0.86 ±0.15 (10)	0.67 — (2)
37 38	1 66 ±0.30 (13)	1 04 ±0.19 (10)	1.87 ±0.36 (10)	1.35 ±0.[illegible]7 (6)	—
39–40	2.98 ±0.69 (17)	0.84 — (1)	[illegible].04 ±0.63 (3)	—	—
41–4[illegible]	3 66 ±0.51 (4)	—	—	—	—

Staining with meconium did not seem to affect the levels significantly

Alkaline phosphatases in amniotic fluid

The alkaline phosphatase activity showed an increase with advancing pregnancy and for normal pregnancies the increase was probably significant from the 35th–36th week to the 39th–40th week (Table IV). Also for Rh group II the rise was probably significant from the 33rd–34th to the 37th–38th week. *The few values in Rh group IV* fell into the lower range of the other groups. The differences for the other groups were not significant. Nonimmunized patients with hepatosis gravidarum had a tendency to low values compared with the normals. Samples from patients with hydramnios or preeclampsia gave values within the normal range. Samples contaminated by visible amounts of meconium showed a very high activity exceeding normal levels 20–400 times.

In normal pregnancies a probably significant correlation was found between the alkaline phosphatase activities in amniotic fluid and maternal serum ($r = 0\ 37$ $n = 30$).

Multiple regression analysis

The analysis was performed with the variables listed in Table V When CI was the dependent variable highly significant relationships were found with the total protein levels in amniotic fluid, with the alkaline phosphatase activity in amniotic fluid, significant relationships with the *blood factor 0 of the mother* and with the cord serum bilirubin concentration. A probably significant relationship existed with the blood factor A of the mother (Fig 8).

Cord blood haemoglobin values were probably significantly influenced by the clinical classification group and by blood factor 0 of the infant (Fig 9).

Cord serum bilirubin concentrations were influenced to a highly significant degree by the clinical group and the Rh factor of the infant. Significant relationships were found with CI

values and total protein concentrations. Probably significant relationships were found with the week of pregnancy to blood factor 0 of the infant and Rh factor of mother (Fig. 10)

The amniotic fluid protein content had highly significant relationships with the week of pregnancy and CI values. Significant influences were exercised by order of pregnancy and cord serum bilirubin values. A probably significant relationship occurred with blood factor 0 of the mother (Fig. 11)

Alkaline phosphatase activity had a highly significant connection with the CI values and a probably significant connection with the week of pregnancy (Fig. 12).

The acid phosphatase activity was not found to be significantly influenced by any of the studied variables. The regression coefficients and their F-values are given in the figures for the variables found to have significant effects

Amniotic fluid total protein and the order of pregnancy

To test whether pregnancy order might influence the amniotic fluid protein concentrations the total number of late normal pregnancies was analysed. Linear regression analysis of the values from gravidae I–II in the weeks 27–43 gave the equation $y = 1032.07 - 20.85\ x\ (n = 36)$. The corresponding equation for gravidae ≥ III was $y = 654.56 - 10.69\ x (n = 36)$ y was then mg protein/100 ml and x the duration of pregnancy in weeks. The slope of the regression lines was significantly different This indicated that the decrease of the protein concentration with advancing pregnancy was slower for the patients with higher order of pregnancy. The regression lines cut each other in the 37th week.

DISCUSSION

No general agreement exists concerning the classification of pregnancies complicated by isoimmunization. The model chosen in this study also takes into account the number of exchange transfusions to the infants. The same principle has been used by others even if the criteria of classification have been somewhat different (8, 11 ?7 3, 40 41). The limit between cases with slight and moderate haemolytic disease (Rh groups I and II) ... [illegible] a haemoglobin value

Table V *Variables used in multiple stepwise regression analysis*

Code	Variable	Mean ± S.D
ID	Clinical isoimmunization group	– –
PW	Week of pregnancy	35.70 ± 2.40
PO	Order of pregnancy	3.06 ± 1.22
S	Sex of infant (male +1 female −1)	−0.09 –
AI	Blood factor A infant (present +1 absent −1)	0.05 –
BI	Blood factor B infant (present +1 absent −1)	−0.66 –
OI	Blood factor 0 infant (present +1 absent −1)	−0.31 –
RhI	Rh factor of infant (positive +1 negative −1)	0.65 –
MA	Age of mother	28.64 ± 5.39
AM	Blood factor A of mother (present +1 absent −1)	0.23 –
BM	Blood factor B of mother (present +1 absent −1)	−0.60 –
OM	Blood factor 0 of mother (present +1 absent −1)	−0.32 –
RhM	Rh factor of mother (positive +1 negative −1)	−0.79 –
CI	Amniotic fluid CI	58.01 ± 60.08
Hbl	Cord blood haemoglobin, g/100 ml	13.21 ± 4.26
Bil	Cord serum bilirubin, mg/100 ml	3.26 ± 1.90
TP	Amniotic fluid total protein mg/100 ml	?91.27 ± 107.34
Ac	Acid phosphatase activity μmol/ml,h	0.20 ± 0.15
Alk	Alkaline phosphatase activity μmol/ml,h	1.48 ± 2.37

of 12.1/100 ml because in the present series of normal pregnancies the mean cord blood haemoglobin value was 15.7 ± 3.6 (2 S.D.) g/100 ml (n = 55). The haemoglobin value 8.1 g/100 ml was selected as the lower limit for cases with moderate disease as all cases with perinatal death due to erythroblastosis alone had cord blood haemoglobin values below this limit.

It was not the aim of the present study to evaluate the clinical results of our management of the Rh-isoimmunized patients. However it may be pointed out that the overall perinatal mortality rate in this series was 12.7% (30 of 237 cases).

Table IV *Alkaline phosphatase in amniotic fluid*

Activity expressed as μmol α naphtol liberated per ml and hour. Means±S.E.M. Figures within brackets indicate number of samples

Pregnancy week	Normal pregnancies	Rh group I	Rh group II	Rh group III	Rh group IV
27–28	0.53 — (1)	—	—	1.96 — (2)	—
29–30	0.63 — (1)	—	—	1.21 ±0.40 (5)	—
31–32	—	—	0.70 ±0.23 (4)	0.91 ±0.39 (5)	0.25 — (1)
33–34	0.78 ±0.22 (3)	0.75 — (2)	0.84 ±0.12 (10)	0.75 ±0.13 (8)	0.32 — (2)
35–36	0.93 ±0.17 (11)	1.16 ±0.21 (8)	1.26 ±0.21 (12)	0.66 ±0.15 (10)	0.67 — (2)
37–38	1.66 ±0.30 (13)	1.04 ±0.19 (10)	1.87 ±0.36 (10)	1.35 ±0.27 (6)	—
39–40	2.98 ±0.69 (17)	0.84 — (1)	2.04 ±0.63 (3)	—	—
41–42	3.66 ±0.51 (4)	—	—	—	—

Staining with meconium did not seem to affect the levels significantly.

Alkaline phosphatases in amniotic fluid

The alkaline phosphatase activity showed an increase with advancing pregnancy and for normal pregnancies the increase was probably significant from the 35th–36th week to the 39th–40th week (Table IV). Also for Rh group II the rise was probably significant from the 33rd–34th to the 37th–38th week. The few values in Rh group IV fell into the lower range of the other groups. The differences for the other groups were not significant. Nonimmunized patients with hepatosis gravidarum had a tendency to low values compared with the normals. Samples from patients with hydramnios or preeclampsia gave values within the normal range. Samples contaminated by visible amounts of meconium showed a very high activity exceeding normal levels 20–400 times.

In normal pregnancies a probably significant correlation was found between the alkaline phosphatase activities in amniotic fluid and maternal serum ($r = 0.37$, $n = 30$).

Multiple regression analysis

The analysis was performed with the variables listed in Table V. When CI was the dependent variable highly significant relationships were found with the total protein levels in amniotic fluid, with the alkaline phosphatase activity in amniotic fluid, significant relationships with the blood factor 0 of the mother and with the cord serum bilirubin concentration. A probably significant relationship existed with the blood factor A of the mother (Fig. 8).

Cord blood haemoglobin values were probably significantly influenced by the clinical classification group and by blood factor 0 of the infant (Fig. 9).

Cord serum bilirubin concentrations were influenced to a highly significant degree by the clinical group and the Rh factor of the infant. Significant relationships were found with CI

values and total protein concentrations. Probably significant relationships were found with the week of pregnancy to blood factor 0 of the infant and Rh factor of mother (Fig. 10).

The amniotic fluid protein content had highly significant relationships with the week of pregnancy and CI values. Significant influences were exercised by order of pregnancy and cord serum bilirubin values. A probably significant relationship occurred with blood factor 0 of the mother (Fig. 11).

Alkaline phosphatase activity had a highly significant connection with the CI values and a probably significant connection with the week of pregnancy (Fig. 12).

The acid phosphatase activity was not found to be significantly influenced by any of the studied variables. The regression coefficients and their F values are given in the figures for the variables found to have significant effects.

Amniotic fluid total protein and the order of pregnancy

To test whether pregnancy order might influence the amniotic fluid protein concentrations the total number of list normal pregnancies was analysed. Linear regression analysis of the values from gravidae I–II in the weeks 27–41 gave the equation $y = 1032.07 - 20.85x$ ($n = 36$). The corresponding equation for gravidae ≥ III was $y = 654.56 - 10.69x$ ($n = 56$); y was then mg protein/100 ml and x the duration of pregnancy in weeks. The slope of the regression lines was significantly different. This indicated that the decrease of the protein concentration with advancing pregnancy was slower for the patients with higher order of pregnancy. The regression lines cut each other in the 37th week.

Table V *Variables used in multiple stepwise regression analysis*

Code	Variable	Mean ± S.D
ID	*Clinical isoimmunization group*	– –
PW	Week of pregnancy	35.70 ± 2.40
PO	Order of pregnancy	3.06 ± 1.22
S	Sex of infant (male +1 female −1)	−0.09 –
AI	Blood factor A infant (present +1 absent −1)	0.05 –
BI	Blood factor B infant (present +1 absent −1)	−0.66 –
OI	Blood factor 0 infant (present +1 absent −1)	−0.31 –
RhI	Rh factor of infant (positive +1 negative −1)	0.65 –
MA	Age of mother	28.94 ± 5.29
AM	Blood factor A of mother (present +1 absent −1)	0.22 –
BM	Blood factor B of mother (present +1 absent −1)	0.80 –
OM	Blood factor 0 of mother (present +1 absent −1)	−0.52 –
RhM	Rh factor of mother (positive +1 negative −1)	−0.79 –
CI	Amniotic fluid CI	58.01 ± 60.09
HbI	Cord blood haemoglobin, g/100 ml	13.21 ± 4.26
BI	Cord serum bilirubin, mg/100 ml	3.26 ± 1.90
TP	Amniotic fluid total protein mg/100 ml	291.27 ± 107.34
Ac	Acid phosphatase activity μmol/ml/h	0.20 ± 0.15
Alk	Alkaline phosphatase activity μmol/ml/h	1.49 ± 2.37

DISCUSSION

No general agreement exists concerning the classification of pregnancies *complicated by* immunization. The model chosen in this study also takes into account the number of exchange transfusions to the infants. The same principle has been used by others even if the criteria of classification has been somewhat different (8, 11, 17, 19, 40, 41). The limit between cases with slight and moderate haemolytic disease (Rh groups I and II) was fixed at a haemoglobin value of 11.1/100 ml because *in the present series of* normal pregnancies the mean cord blood haemoglobin value was 15.7 ± 3.6 (± S.D.) g/100 ml ($n = 55$). The haemoglobin value 8.1 g/100 ml was selected as the lower limit for cases with *moderate disease as all cases with perinatal death* due to erythroblastosis alone had cord blood haemoglobin values below this limit.

It was not the aim of the present study to evaluate the clinical results of our management of the Rh-isoimmunized patients. However it may be pointed out that the overall perinatal mortality rate in this series was 12.7% (30 of 237 cases).

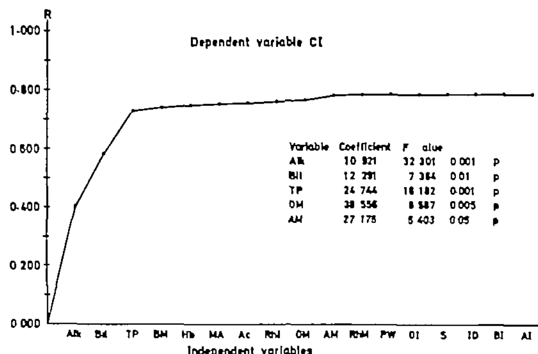


Fig 8 Multiple regression analysis. Variables with significant influence upon dependent variable are listed in the figure.

If only cases were included where Rh disease was the main cause of foetal death the perinatal death rate was 8.9% (21 of 237) Seven foetuses died in utero.

The original method of CI determination has been used in this study as it is rapid and simple. Various modifications have been devised to make the method more specific for bilirubin (7 12, 14 37). They seem to offer only marginal advantages over the original method. Extraction of bilirubin

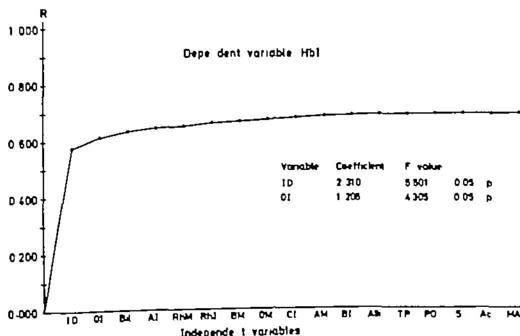


Fig 9 Multiple regression analysis. Variables with significant influence upon dependent variable are listed in the figure.

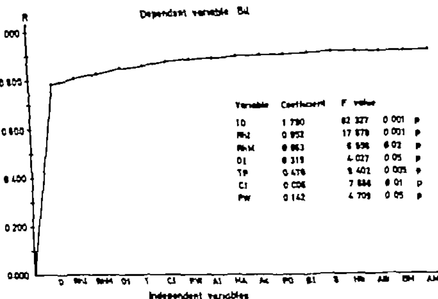


Fig. 10. Multiple regression analysis. Variables with significant influence upon dependent variable are listed in the figure.

with chloroform is supposed to give more reliable determinations in specimens contaminated by blood or meconium (9 18). On the other hand bilirubin seems to be responsible only for a part of the ΔE_{450} peak and the role of other pigments is not yet fully established (36). Spectrophotometric determinations of bile pigments seems to be superior to chemical analysis (24 25 34).

Clinically it is most important to be able to distinguish patients with serious or very serious

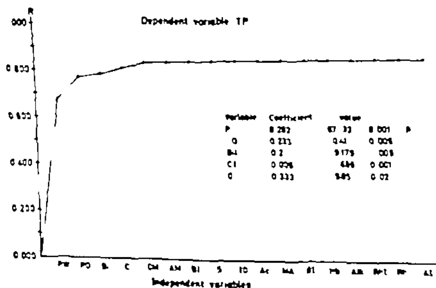


Fig. 11. Multiple regression analysis. Variables with significant influence upon dependent variable are listed in the figure.

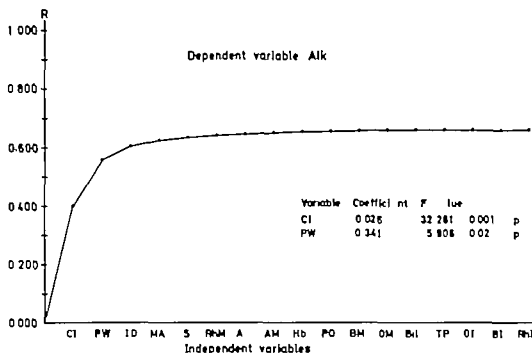


Fig 12 Multiple regression analysis. Variables with significant influence upon dependent variable are listed in the figure.

disease (groups III and IV) from patients with mild to moderate disease The former will require early delivery (or intrauterine transfusion) and close cooperation with the paediatricians for proper postnatal care without delay There is a considerable overlap in CI values between the clinical groups. The tendency to increasing values in the individual case is, however especially pronounced in pregnancies with very serious disease Repeated sampling will thus help to detect those cases. 16 of the 53 cases with serious disease (group III) had CI values in the lower part of the middle zone or in the lowest zone but only two of these infants had a cord blood haemoglobin value below 8 1 g/100 ml. Thus in practice there seems to be little risk of overlooking cases with serious anaemia.

From a clinical standpoint it is also important to be able to separate a pregnancy with a healthy Rh negative fetus (heterozygotic father) from a pregnancy with an affected fetus. It is clearly not possible to make a safe discrimination between normal cases and cases with slight to moderate disease (Rh group I and II) after the 35th or 36th week due to the considerable overlap of the CI values during the last weeks of pregnancy The incidence of increasing CI values on repeated sampling is evidently about the same in normal pregnancies as in cases with moderate disease. The tendency towards increasing values seems to be especially pronounced in late pregnancy Taking normal cases and cases with mild to moderate disease (Rh groups I and II) together CI values above the lower half of the middle zone occurred in 19 cases of 202 (9 4%) which thus represents a figure for false predictions of severe disease

A rather good correlation existed between CI values and cord blood haemoglobin levels for pregnancies complicated by severe haemolytic disease but not for pregnancies with slight to moderate disease. It thus seems evident that the CI values and the trends in repeated samples observed before the 37th week have greater predictive value than later observations. From the 37th week onwards the CI values alone are of little importance in evaluating the status of pregnancies with slight to moderate haemolytic disease

The poor correlation between the CI values and the cord serum bilirubin concentrations in cases with severe haemolytic disease is somewhat surprising. This may indicate that the transfer of bilirubin into the amniotic fluid is disturbed by the haemolytic process. Another possibility is that other pigments are responsible for an important part of the optical density peak in these cases. Yet other investigators have found that the major

part of the optical density is due to indirect react ing bilirubin in cases affected by haemolytic disease (29 36, 44). The better correlation between CI and cord serum bilirubin in cases with slight to moderate disease would indicate that the haemolytic disease in itself influences the transport mechanism of bilirubinlike pigments to and from the amniotic fluid.

Variations in maternal serum bilirubin concentrations within normal range (0.1–1.0 mg/100 ml) does not seem to affect the CI values in normal pregnancies but in cases with hepatosis gravidarum there is a clear correlation between the CI alues nd maternal serum bilirubin levels. In the present series only a few values for cord serum bilirubin in these cases were available and they all fell within the range for normal infants. This would indicate that maternal bilirubin elevation influences the amniotic fluid bilirubin directly and not as any increase of fetal bilirubin levels. That maternal bilirubin elevation raises the CI values has been demonstrated earlier (15 22, 25 28).

Apart from the influence of an elevated maternal bilirubin level the present series also illustrates other possible sources of error in CI determinations The elevated alues found in the t o cases ith obstruction of the fetal oesophagus nd small intestine indicate that fetal swallowing and elimination in the intestine may play an important role in removing bile pigments from the amniotic fluid. Contamination of amniotic fluid ith nsible amount of meconium was observed in fiv of the nonimmunized patients with hepatosis gravidarum and also in two immunized pa tient ith laboratory tests indicating hepatosis No other signs of fetal intrauterine asphyxia were observed in these cases. A connection between maternal hyperbilirubinaemia and meconium staining has also been observed by others (46) Discolouration of the amniotic fluid and an ele ted CI observed in the present series in one patient with a small placental haemorrhage.

Both in normal pregnancies and in Rh-immunized patients with affected fetuses (group I IV) a corr lation exists between the CI alues and the mniotic fluid total protein content. The correlation is weaker during the last few weeks of pregnancy and especially so for cases ith slight to moderate haemolytic disease. The correlation may be due to the fact that bilirubin usually is bound to protein, mostly albumin, in the amniotic fluid (44). The mean levels for both CI and total protein have a very similar distribution during the course of normal pregnancies (1 23 40). The higher correlation in cases with serious haemolytic disease may be due to more complete saturation of the protein with bilirubin. In cases with slight disease a tendency to high protein levels has also been observed (23).

It has been suggested that the ratio between bilirubin and protein concentrations in amniotic fluid would give a more reliable prediction of the fetal condition in haemolytic disease than the bilirubin or CI values alone (11 32, 33). In the present study no better discrimination between the clinical groups was found by this method. This is in accordance with the results of other investigators (39 43). The ratio seems to ha e a somewhat higher correlation with cord serum bilirubin levels then the CI determinations alone. Noteworthy is the rapid decline in the ratio during the last few weeks of pregnancy except in the most serious cases of haemolytic disease.

It has been suggested that determination of bile pigments in amniotic fluid would be of value in estimating fetal maturity as in nonimmunized pregnancies low levels dominate during the last few weeks of pregnancy (29 31 38, 45). The results of the present study indicate that a CI value of 10 or lower is always associated with an infant whose birth weight is at least 2 500 g and in most cases the birth weight will exceed 3 000 g On the other hand only about one third of infants with a birth weight exceeding 500 g will have CI alue of 10 or lower just prior to delivery

The pattern of distribution of the levels of amniotic fluid progesterone CI and total protein are rather similar in normal lat pregnancies. This may e plain the correlation between the progesterone levels, CI and total protein When shorter periods of gestation are studied the correlation between progesterone and total protein weakens (20).

A carrier function of the proteins for bile pigments and progesterone may also e plain the correlations. The connection between progesterone nd protein content seems to be weaker in Rh-isoimmunization with an affected fetus (groups I IV) The poor correlation between progesterone and cord blood haemoglobin concentrations was observed previously (?1). Progesterone determina-

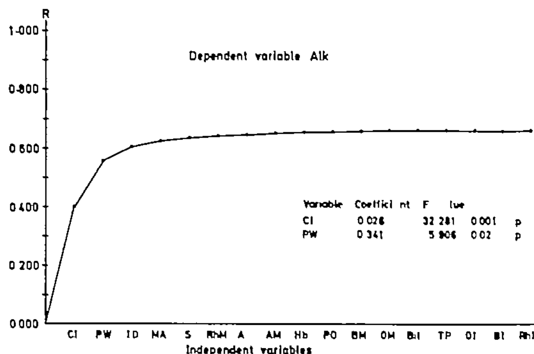


Fig 12 Multiple regression analysis. Variables with significant influence upon dependent variable are listed in the figure

disease (groups III and IV) from patients with mild to moderate disease The former will require early delivery (or intrauterine transfusion) and close cooperation with the paediatricians for proper postnatal care without delay There is a considerable overlap in CI values between the clinical groups. The tendency to increasing values in the individual case is, however especially pronounced in pregnancies with very serious disease. Repeated sampling will thus help to detect those cases. 16 of the 53 cases with serious disease (group III) had CI values in the lower part of the middle zone or in the lowest zone but only two of these infants had a cord blood haemoglobin value below 8.1 g/100 ml Thus in practice there seems to be little risk of overlooking cases with serious anaemia.

From a clinical standpoint it is also important to be able to separate a pregnancy with a healthy Rh negative fetus (heterozygotic father) from a pregnancy with an affected fetus. It is clearly not possible to make a safe discrimination between normal cases and cases with slight to moderate disease (Rh group I and II) after the 35th or 36th week due to the considerable overlap of the CI values during the last weeks of pregnancy The incidence of increasing CI values on repeated sampling is evidently about the same in normal pregnancies as in cases with moderate disease The tendency towards increasing values seems to be especially pronounced in late pregnancy Taking normal cases and cases with mild to moderate disease (Rh groups I and II) together CI values above the lower half of the middle zone occurred in 19 cases of 20? (9 4) which thus represents a figure for false predictions of severe disease.

A rather good correlation existed between CI values and cord blood haemoglobin levels for pregnancies complicated by severe haemolytic disease but not for pregnancies with slight to moderate disease. It thus seems evident that the CI values and the trends in repeated samples observed before the 37th week have greater predictive value than later observations. From the 37th week onwards the CI values alone are of little importance in evaluating the status of pregnancies with slight to moderate haemolytic disease.

The poor correlation between the CI values and the cord serum bilirubin concentrations in cases with severe haemolytic disease is somewhat surprising This may indicate that the transfer of bilirubin into the amniotic fluid is disturbed by the haemolytic process. Another possibility is that other pigments are responsible for an important part of the optical density peak in these cases. Yet other investigators have found that the major

part of the optical density is due to indirect reacting bilirubin in cases affected by haemolytic disease (29 36, 44). The better correlation between CI and cord serum bilirubin in cases with slight to moderate disease would indicate that the haemolytic disease in itself influences the transport mechanism of bilirubinlike pigments to and from the amniotic fluid.

Variations in maternal serum bilirubin concentrations within normal range (0 1–1 0 mg/100 ml) does not seem to affect the CI values in normal pregnancies but in cases with hepatosis gravidarum there is a clear correlation between the CI values and maternal serum bilirubin levels. In the present series only a few values for cord serum bilirubin in these cases were available and they all fall within the range for normal infants. This would indicate that maternal bilirubin elevation influences the amniotic fluid bilirubin directly and not via any increase of fetal bilirubin levels. That maternal bilirubin elevation raises the CI values has been demonstrated earlier (15 22, 25 28).

Apart from the influence of an elevated maternal bilirubin level the present series also illustrates other possible sources of error in CI determinations. The elevated values found in the [illegible] cases with obstruction of the fetal oesophagus and small intestine indicate that fetal swallowing and elimination via the intestine may play an important role in removing bile pigments from the amniotic fluid. Contamination of amniotic fluid with visible amounts of meconium was observed in five of the nonimmunized patients with hepatosis gravidarum and also in two immunized patients with laboratory tests indicating hepatosis. No other signs of fetal intrauterine asphyxia were observed in these cases. A connection between maternal hyperbilirubinaemia and meconium staining has also been observed by others (46). Discolouration of the amniotic fluid and an elevated CI was observed in the present series in one patient with small placental haemorrhage.

Both in normal pregnancies and in Rh-isoimmunized patients with affected fetuses (group I IV) correlation exists between the CI values and the amniotic fluid total protein content. The correlation is weaker during the last few weeks of pregnancy and especially so for cases with slight to moderate haemolytic disease. The correlation may be due to the fact that bilirubin usually is bound to protein, mostly albumin, in the amniotic fluid (44). The mean levels for both CI and total protein have a very similar distribution during the course of normal pregnancies (1 23 40). The higher correlation in cases with serious haemolytic disease may be due to more complete saturation of the protein with bilirubin. In cases with slight disease a tendency to high protein levels has also been observed (23).

It has been suggested that the ratio between bilirubin and protein concentrations in amniotic fluid would give a more reliable prediction of the fetal condition in haemolytic disease than the bilirubin or CI values alone (11 32, 33). In the present study no better discrimination between the clinical groups was found by this method. This is in accordance with the results of other investigators (39 43). The ratio seems to have a somewhat higher correlation with cord serum bilirubin levels then the CI determinations alone. Noteworthy is the rapid decline in the ratio during the last few weeks of pregnancy except in the most serious cases of haemolytic disease.

It has been suggested that determination of bile pigments in amniotic fluid would be of value in estimating fetal maturity as in nonimmunized pregnancies low levels dominate during the last few weeks of pregnancy (29 31 38, 45). The results of the present study indicate that a CI value of 10 or lower is always associated with an infant whose birth weight is at least 2 500 g and in most cases the birth weight will exceed 3 000 g. On the other hand only about one third of infants with a birth weight exceeding 500 g will have a CI value of 10 or lower just prior to delivery

The pattern of distribution of the levels of amniotic fluid progesterone CI and total protein are rather similar in normal late pregnancies. This may explain the correlation between the progesterone levels, CI and total protein When shorter periods of gestation are studied the correlation between progesterone and total protein weakens (20)

A carrier function of the proteins for bile pigment and progesterone may also explain the correlations. The connection between progesterone and protein content seems to be weaker in Rh-isoimmunization with an affected fetus (groups I IV) The poor correlation between progesterone and cord blood haemoglobin concentrations was observed previously (21) Progesterone determina-

tions thus will be of limited value in the clinical management of Rh-isoimmunized patients. The few reliable oestrogen determinations in this study indicate a tendency to low values in cases with very serious haemolytic disease.

Determination of acid phosphatase activity in amniotic fluid does not seem to give any clinically important information with respect to Rh-isoimmunized patients. To some extent the activity seems to be related to gestational age.

Alkaline phosphatase activity also was found to differ very little between the clinical groups. The increase with gestational age was more pronounced than for acid phosphatase. Alkaline phosphatase in amniotic fluid has previously been studied by only few authors. *Lapan and Friedman* (26) found values around 50% of the activity in maternal serum at term in normal pregnancies. *Geyer and Schneider* (16) found somewhat lowered values for both acid and alkaline phosphatase in pregnancies complicated by erythroblastosis. The high alkaline phosphatase activity of meconium was noted also by them. Determination of alkaline phosphatase activity ought to be a sensitive test for the detection of even small amounts of meconium contamination. The present study gives no explanation as to the origin of the phosphatases, this will require further studies of isoenzymes. In normal pregnancies a probably significant correlation existed between the alkaline phosphatase activity in amniotic fluid and maternal serum. Since the increase of maternal serum alkaline phosphatase activity during pregnancy is due exclusively to placental alkaline phosphatase (3) the possibility that amniotic fluid in late pregnancy contains placental phosphatases should be examined. The maternal liver phosphatase does not apparently influence the level in amniotic fluid because in cases of hepatosis gravidarum the high activity usually found in maternal serum did not give any corresponding elevation in amniotic fluid activity

The multiple regression analysis was made with the purpose of revealing any unknown connections between the variables studied. The series was rather small and thus the results must be interpreted with caution. The concentrations of haemoglobin and bilirubin in cord blood are of special interest as they are the most important indicators of fetal status at birth in pregnancies complicated by haemolytic disease One interesting finding in this study is therefore the poor correlation tween the cord blood haemoglobin concentratio and the other variables. Cord blood bilirubin more influenced by the other factors. factor 0 in the fetus seems to be positively related with both cord blood haemoglobin ar *bilirubin concentrations. It may be that this* factor in the fetus gives a better resistance haemolytic disease. The material contains in several infants of blood group 0 with both h cord blood haemoglobin and bilirubin levels. Th the AB0 factors of mother and child influen the effects of isoimmunization is already kno (35) If mother and child are incompatible in ABO system the effects of isoimmunization are di minished. This *may explain the effects in this* series of the maternal blood factors A and 0 o, the CI values and the total protein levels in am niotic fluid. It has been shown previously the amniotic fluid total protein levels usually rather low in cases with moderate to severe molytic disease (23). This may also explain negative correlation between the cord serum bili rubin and total protein concentrations. That Rh factors of mother and fetus influence the serum bilirubin level is only to be expected. T CI values were dependent on cord serum bi rubin concentrations This is in contrast with whole series where this correlation was weak. *It is also interesting to note the strong* correlations of CI with total protein levels and alkaline phosphatase activity The correlation between CI and amniotic fluid total protein levels was also found in the series as a whole. In the case of alkaline phosphatase, contamination with small amounts of meconium cannot be excluded and the correlation may thus be spurious. The series contained a few cases with high alkaline phosphatase activity but without visible meconium contamination Differences in membrane permeability affecting both bilirubin-like substances and alkaline phosphatase is also a possibility The total protein level in amniotic fluid seems to be influenced by the order of pregnancy This was also found in the total series of normal pregnancies where multigravidae had higher protein concentrations during the last few weeks of pregnancy This may have some connection with the well known fact that infant birth weight tends to increase in subsequent pregnancies.

From this series it seems clear that the CI is a

rather inaccurate indicator of the fetal haemoglobin level. The influence of the AB0 blood groups indicates that genetic factors may be of importance in modifying the effects of haemolytic disease and the passage of substances to and from the amniotic fluid. Of the factors covered by this study CI is still the best one to use in the management of isoimmunized patients in spite of all its limitations. The great variability of the CI measurements, especially late in pregnancy makes it necessary to use the CI method in combination with other information.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Professor Lars Beckman for kind advice and help with the phosphatase determinations and the multiple regression analyses.

Dr Elof Johansson and the personnel at the Pituitary Laboratory for Reproduction Research have been very helpful in performing the steroid analyses and preparing the manuscript.

This study has been supported by the University of Uppsala.

REFERENCES

1 Bartsch, F. K., Persson, E. F. S., Carlström, E. & Eriksson, B. Der Pigmentindex des Fruchtwassers während der normalen Schwangerschaft. Gynaecologia *168* 171, 1969.

2 Beckman, G. Placental alkaline phosphatase, relation between phenotype and enzyme activity. Hum Hered *20* 74, 1970.

3 Beckman, L., Beckman, G. & Magnusson, S. S. Factors influencing the levels of the alkaline phosphatases in maternal serum and cord serum. Hum Hered *21* 49, 1971.

4 Bevis, D. C. A. Composition of liquor amnii in haemolytic disease of the newborn. Lancet ii 443, 1950.

5 — Blood pigments in haemolytic disease of the newborn. J Obst Gynaec Brit Emp *63* 68, 1956.

6 BMD Biomedical Computer Programs. Health Sciences Computing Facility, University of California, Los Angeles, 1965.

7 Bowers, R. W. Instrument News *16* ... 1965. Perkin Elmer Corporation.

8 Bowes, W. A., Drose, V. E. & Bruns, P. D. Amniocentesis and intrauterine fetal transfusion in erythroblastosis. Am J Obstet Gynec *93* 822, 1965.

9 Brazie, J. V., Bowes, W. A. & Ibbott, F. A. An improved rapid procedure for the determination of amniotic fluid bilirubin and its use in the prediction of the course of Rh-sensitized pregnancies. Am J Obstet Gynec *104* 80, 1969.

10 Brown, J. B., MacLeod, S. C., Macnaughtan, C., Smith, M. A. & Smyth, B. A rapid method for estimating oestrogens in urine using a semi-automatic extractor. J Endocrin *42* 5, 1968.

11. Cherry, S. H., Kochwa, S. & Rosenfield, R. E. Bilirubin–Protein ratio in amniotic fluid as an index of the severity of erythroblastosis fetalis. Obstet Gynec 26, 826, 1965.

12. Clayton, E. M., Walker, D. H. & Foster, E. B. The significance of hemepigments in amniotic fluid. Obstet Gynec *34* 641, 1969.

13. Efroymson, M. A. Multiple regression analysis. Mathematical methods for digital computers, part V (ed. A. Ralston & H. S. Wilf). J Wiley & Sons, New York, 1964.

14. Fleming, E. A. & Woolf, A. J.: A spectrophotometric method for the quantitative estimation of bilirubin in liquor amnii. Clin Chim Acta *12* 67, 1965.

15 Fort, A. T. Prenatal intrusion into the amnion. Am J Obstet Gynec *110* 432, 1971.

16. Geyer, H. & Scheider, I. Enzyme im Fruchtwasser. Z Klin Chem Klin Biochem *8* 141, 1970.

17 Haemmerli, U. P. Jaundice during pregnancy. Acta Med Scand *179* Suppl 444, 1966.

18. Hablasky, V. & Krumholz, B. A. Amniotic fluid analysis in erythroblastosis fetalis. III. The chloroform extract and its relationship to the log OD 450. Am J Obstet Gynec *106* 1209, 1970.

19 Ikonen, E. Jaundice in late pregnancy. Acta Obstet Gynec Scand *43* Suppl. 5, 1964.

20 Johansson, E. D. B. & Jonasson, L.-E. Progesterone levels in amniotic fluid and plasma from women. I. Levels during normal pregnancy. Acta Obstet Gynec Scand *50* 339, 1971.

21 Jonasson, L. E. & Johansson, E. D. B. Progesterone levels in amniotic fluid and plasma from women. II. Levels during pregnancies complicated by Rh-isoimmunization or hepatosis gravidarum. Acta Obstet Gynec Scand 50 345, 1971.

22. Jonasson, L. E. & Persson, B. H. Spectrophotometric screening of amniotic fluid in haemolytic disease and hepatosis gravidarum. Acta Obstet Gynec Scand *47* 300, 1968.

23 Jonasson, L. E. Total protein content in amniotic fluid from normal pregnancies and in pregnancies complicated by Rh-isoimmunization. Acta Obstet Gynec Scand *51* 187, 1972.

24 Kuah, M. Y., Marko, J. & Leth, H. Bilirubin estimation in amniotic fluid. Am J Obstet Gynec *108* 992, 1970.

25 Kubli, F. Die antenatale Diagnose des Rhesusbedingten Morbus Haemolyticus und die vorzeitige Beendigung der Schwangerschaft. Bibl Gynaec *38* 30, 1966.

26. Lapan, B. & Friedman, M. M. Enzymes in the amniotic fluid and maternal serum. Am J Obstet Gynec *83* 1337, 1962.

27 Liley, A. W. Liquor amnii analysis in the management of the pregnancy complicated by rhesus sensitization. Am J Obstet Gynec *82* 1359, 1961.

28 — Errors in the assessment of hemolytic disease from amniotic fluid. Am J Obstet Gynec *86* 485, 1963.

9 [illegible]

tions thus will be of limited value in the clinical management of Rh-isoimmunized patients. The few reliable oestrogen determinations in this study indicate a tendency to low values in cases with very serious haemolytic disease.

Determination of acid phosphatase activity in amniotic fluid does not seem to give any clinically important information with respect to Rh-isoimmunized patients. To some extent the activity seems to be related to gestational age.

Alkaline phosphatase activity also was found to differ very little between the clinical groups. The increase with gestational age was more pronounced than for acid phosphatase. Alkaline phosphatase in amniotic fluid has previously been studied by only few authors. *Lapan and Friedman* (26) found values around 50% of the activity in maternal serum at term in normal pregnancies. *Geyer and Schneider* (16) found somewhat lowered values for both acid and alkaline phosphatase in pregnancies complicated by erythroblastosis. The high alkaline phosphatase activity of meconium was noted also by them. Determination of alkaline phosphatase activity ought to be a sensitive test for the detection of even small amounts of meconium contamination. The present study gives no explanation as to the origin of the phosphatases, this will require further studies of isoenzymes. In normal pregnancies a probably significant correlation existed between the alkaline phosphatase activity in amniotic fluid and maternal serum. Since the increase of maternal serum alkaline phosphatase activity during pregnancy is *due exclusively* to placental alkaline phosphatase (3) the possibility that amniotic fluid in late pregnancy contains placental phosphatases should be examined. The maternal liver phosphatase does not apparently influence the level in amniotic fluid because in cases of hepatosis gravidarum the high activity usually found in maternal serum did not give any corresponding elevation in amniotic fluid activity

The multiple regression analysis was made with the purpose of revealing any unknown connections between the variables studied. The series was rather small and thus the results must be interpreted with caution. The concentrations of haemoglobin and bilirubin in cord blood are of special interest as they are the most important indicators of fetal status at birth in pregnancies complicated by haemolytic disease. One interesting finding in this study is therefore the poor correlation b tween the cord blood haemoglobin concentratio and the other variables. Cord blood bilirubin more influenced by the other factors. Bloo factor 0 in the fetus seems to be positively cor related with both cord blood haemoglobin an bilirubin concentrations. It may be that this bloo factor in the fetus gives a better resistance agains haemolytic disease. The material contains in fac several infants of blood group 0 with both hig cord blood haemoglobin and bilirubin levels. Th the AB0 factors of mother and child influenc the effects of isoimmunization is already know (35). If mother and child are incompatible in th AB0 system the effects of isoimmunization are di minished. This may explain the effects in this se ries of the maternal blood factors A and 0 upor the CI values and the total protein levels in am niotic fluid. It has been shown previously tha the amniotic fluid total protein levels usually are rather low in cases with moderate to severe hae molytic disease (23). This may also explain the negative correlation between the cord serum bilirubin and total protein concentrations. That the Rh factors of mother and fetus influence the cord serum bilirubin level is only to be expected. The CI values were dependent on cord serum bilirubin concentrations. This is in contrast with the whole series where this correlation was rather weak. It is also interesting to note the strong correlations of CI with total protein levels and alkaline phosphatase activity The correlation between CI and amniotic fluid total protein levels was also found in the series as a whole. In the case of alkaline phosphatase contamination with small amounts of meconium cannot be excluded, and the correlation may thus be spurious. The series contained a few cases with high alkaline phosphatase activity but without visible meconium contamination. Differences in membrane permeability affecting both bilirubin-like substances and alkaline phosphatase is also a possibility The total protein level in amniotic fluid seems to be influenced by the order of pregnancy This was also found in the total series of normal pregnancies where multigravidae had higher protein concentrations during the last few weeks of pregnancy This may have some connection with the well known fact that infant birth weight tends to increase in subsequent pregnancies.

From this series it seems clear that the CI is a

rather inaccurate indicator of the fetal haemoglobin level. The influence of the AB0 blood groups indicates that genetic factors may be of importance in modifying the effects of haemolytic disease and the passage of substances to and from the amniotic fluid. Of the factors covered by this study CI is still the best one to use in the management of isoimmunized patients in spite of all its limitations. The great variability of the CI measurements, especially late in pregnancy makes it necessary to use the CI method in combination with other information.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Professor Lars Beckman for kind advice and help with the phosphatase determinations and the multiple regression analyses.

Dr Elof Johansson and the personnel at the Prosanto Laboratory for Reproduction Research have been very helpful in performing the steroid analyses and preparing the manuscript.

This study has been supported by the University of Uppsala.

REFERENCES

1. Bartsch, F. K., Persson, E. F. S., Carlström, E. & Eriksson, R.: Der Pigmentindex des Fruchtwassers während der normalen Schwangerschaft. Gynaecologia *168*: 171, 1969.
2. Beckman, G.: Placental alkaline phosphatase: relation between phenotype and enzyme activity. Hum Hered *20*: 74, 1970.
3. Beckman, L., Beckman, G. & Magnusson, S. S.: Factors influencing the levels of the alkaline phosphatases in maternal serum and cord serum. Hum Hered *1*: 69, 1971.
4. Bevis, D. C. A.: Composition of liquor amnii in haemolytic disease of the newborn. Lancet *2*: 443, 1950.

— Blood pigments in haemolytic disease of the newborn. J Obst Gynaec Brit Emp *63*: 68, 1956.

6. BMD Biomedical Computer Programs, Health Sciences Computing Facility. University of California, Los Angeles, 1965.
7. Bowers, R. W.: Instrument News *16*: —, 1965. Perkin Elmer Corporation.
8. Bowes, W. A., Drose, V. E. & Bruns, P. D.: Amniocentesis and intrauterine *fetal transfusion* in erythroblastosis. Am J Obstet Gynec *93*: 822, 1965.
9. Brans, J. V., Bowes, W. A. & Ibbott, F. A.: An improved rapid procedure for the determination of amniotic fluid bilirubin and its use in the prediction of the course of Rh-sensitized pregnancies. Am J Obstet Gynec *104*: 80, 1969.
10. Brown, J. B., MacLeod, S. C., Macnaughton, C., Smith, M. A. & Smyth, B.: A rapid method for estimating oestrogens in urine using a semi-automatic extractor. J Endocrin *42*: 5, 1968.
11. Cherry, S. H., Kochwa, S. & Rosenfield, R. E.: *Bilirubin–Protein ratio in amniotic* fluid as an index of the severity of erythroblastosis fetalis. Obstet Gynec *26*: 826, 1965.
12. Clayton, E. M., Walker, D. H. & Foster, E. B.: The significance of hemepigments in amniotic fluid. Obstet Gynec *34*: 641, 1969.
13. Efroymson, M. A.: Multiple regression analysis. Mathematical methods for digital computers, part V (ed. A. Ralston & H. S. Wilf), J. Wiley & Sons, New York, 1964.
14. Fleming, E. A. & Woolf, A. J.: A spectrophotometric method for the quantitative estimation of bilirubin in liquor amnii. Clin Chim Acta *12*: 67, 1965.
15. Fort, A. T.: *Prenatal intrusion into the amnion.* Am J Obstet Gynec *110*: 432, 1971.
16. Geyer, H. & Schelder, I.: Enzyme im Fruchtwasser. Z Klin Chem Klin Biochem *8*: 141, 1970.
17. Haemmerli, U. P.: Jaundice during pregnancy. Acta Med Scand *179*: Suppl. 444, 1966.
18. Halitsky, V. & Krumholz, B. A.: Amniotic fluid analysis in erythroblastosis fetalis. III. The chloroform extract and its relationship to the log OD 450. Am J Obstet Gynec *106*: 1209, 1970.
19. Ikonen, E.: *Jaundice in late pregnancy.* Acta Obstet Gynec Scand *43*: Suppl. 5, 1964.
20. Johansson, E. D. B. & Jonasson, L.-E.: Progesterone levels in amniotic fluid and plasma from women. I. Levels during normal pregnancy. Acta Obstet Gynec Scand *50*: 339, 1971.
21. Jonasson, L. E. & Johansson, E. D. B.: Progesterone levels in amniotic fluid and plasma from women. II. Levels during pregnancies complicated by Rh-immunization or hepatosis gravidarum. Acta Obstet *Gynec Scand 50*: *345, 1971.*
22. Jonasson, L.-E. & Persson, B. H.: Spectrophotometric screening of amniotic fluid in haemolytic disease and hepatosis gravidarum. Acta Obstet Gynec Scand *47*: 300, 1968.
23. Jonasson, L.-E.: Total protein content in amniotic fluid from normal pregnancies and in pregnancies complicated by Rh immunization. Acta Obstet Gynec Scand *51*: 187, 1972.
24. Kuh, M. Y., Marko, J. & Leth, H.: Bilirubin estimation in amniotic fluid. Am J Obstet Gynec *106*: 992, 1970.
25. Kubli, F.: Die antenatale Diagnose des Rhesusbedingten Morbus Haemolyticus und die vorzeitige Beendigung der Schwangerschaft. Bibl Gynaec *38*: 30, 1966.
26. Lapan, B. & Friedman, M. M.: Enzymes in the amniotic *fluid* and maternal serum. Am J Obstet Gynec *83*: 1337, 1962.
27. Liley, A. W.: Liquor amnii analysis in the management of the pregnancy complicated by *rhesus* sensitization. Am J Obstet Gynec *82*: 1359, 1961.
28. — Errors in the assessment of hemolytic disease from amniotic fluid. Am J Obstet Gynec *86*: 485, 1963.

9 73742

tions thus will be of limited value in the clinical management of Rh-isoimmunized patients. The few reliable oestrogen determinations in this study indicate a tendency to low values in cases with very serious haemolytic disease.

Determination of acid phosphatase activity in amniotic fluid does not seem to give any clinically important information with respect to Rh-isoim munized patients. To some extent the activity seems to be related to gestational age.

Alkaline phosphatase activity also was found to differ very little between the clinical groups. The increase with gestational age was more pronounced than for acid phosphatase. Alkaline phosphatase in amniotic fluid has previously been studied by only few authors. *Lapan and Friedman* (26) found values around 50% of the activity in maternal serum at term in normal pregnancies. *Geyer and Schneider* (16) found somewhat low ered values for both acid and alkaline phosphatase in pregnancies complicated by erythroblastosis. The high alkaline phosphatase activity of me conium was noted also by them. Determination of alkaline phosphatase activity ought to be a sensitive test for the detection of even small amounts of meconium contamination. The present study gives no explanation as to the origin of the phosphatases, this will require further studies of isoenzymes. In normal pregnancies a probably signif icant correlation existed between the alkaline phosphatase activity in amniotic fluid and maternal serum. Since the increase of maternal serum alkaline phosphatase activity during pregnancy is due exclusively to placental alkaline phosphatase (3) the possibility that amniotic fluid in late pregnancy contains placental phosphatases should be examined. The maternal liver phosphatase does not apparently influence the level in amniotic fluid because in cases of hepatosis gravidarum the high activity usually found in maternal serum did not give any corresponding elevation in amniotic fluid activity

The multiple regression analysis was made with the purpose of revealing any unknown connec tions between the variables studied. The series was rather small and thus the results must be inter preted with caution The concentrations of haemoglobin and bilirubin in cord blood are of special interest as they are the most important indicators of fetal status at birth in pregnancies complicated by haemolytic disease. One interesting finding in this study is therefore the poor correlation tween the cord blood haemoglobin concentratic and the other variables. Cord blood bilirubin more influenced by the other factors. B factor 0 in the fetus seems to be positively related with both cord blood haemoglobin bilirubin concentrations. It may be that this bl factor in the fetus gives a better resistance haemolytic disease. The material contains in several infants of blood group 0 with both hig cord blood haemoglobin and bilirubin levels. Th the AB0 factors of mother and child influenc the effects of isoimmunization is already know (35). If mother and child are incompatible in th AB0 system the effects of isoimmunization are di minished. This may explain the effects in this ries of the maternal blood factors A and 0 the CI values and the total protein levels in niotic fluid. It has been shown previously tha the amniotic fluid total protein levels usually ar rather low in cases with moderate to severe hae molytic disease (23). This may also explain th negative correlation between the cord serum bili rubin and total protein concentrations. That Rh factors of mother and fetus influence the serum bilirubin level is only to be expected. T CI values were dependent on cord serum bili rubin concentrations. This is in contrast with whole series where this correlation was weak. It is also interesting to note the s correlations of CI with total protein levels alkaline phosphatase activity The correlation tween CI and amniotic fluid total protein level was also found in the series as a whole. In case of alkaline phosphatase, contamination wi small amounts of meconium cannot be excluded, and the correlation may thus be spurious. T series contained a few cases with high alkal phosphatase activity but without visible meconium contamination. Differences in membrane permeability affecting both bilirubin like substances and alkaline phosphatase is also a possibility The total protein level in amniotic fluid seems to be influenced by the order of pregnancy This was also found in the total series of normal pregnancies where multigravidae had higher protein concentrations during the last few weeks of preg nancy This may have some connection with the well known fact that infant birth weight tends to increase in subsequent pregnancies.

From this series it seems clear that the CI is a

rather inaccurate indicator of the fetal haemoglobin level. The influence of the AB0 blood groups indicates that genetic factors may be of importance in modifying the effects of haemolytic disease and the passage of substances to and from the amniotic fluid. Of the factors covered by this study CI is still the best one to use in the management of isoimmunized patients in spite of all its limitations. The great variability of the CI measurements, especially late in pregnancy makes it necessary to use the CI method in combination with other information.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Professor Lars Beckman for kind advice and help with the phosphatase determinations and the multiple regression analyses.

Dr Elof Johansson and the personnel at the Primate Laboratory for Reproduction Research have been very helpful in performing the steroid analyses and preparing the manuscript.

This study has been supported by the University of Uppsala.

REFERENCES

1 Barsch, F. K., Persson, E. P. S., Carlström, E. & Eriksson, B. Der Pigmentindex des Fruchtwassers während der normalen Schwangerschaft. Gynaecologia *168* 171, 1969.

2 Beckman, G. Placental alkaline phosphatase, relation between phenotype and enzyme activity. Hum Hered 20 74, 1970.

3 Beckman, L., Beckman, G. & Magnusson, S. S. Factors influencing the levels of the alkaline phosphatases in maternal serum and cord serum. Hum Hered *2* 49, 1971.

4 Bevis, D. C. A. Composition of liquor amnii in haemolytic disease of the newborn. Lancet 2 443, 1950.

5 — Blood pigments in haemolytic disease of the newborn. J Obst Gynaec Brit Emp *63* 68, 1956.

6 BMD Biomedical Computer Programs, Health Sciences Computing Facility. University of California, Los Angeles, 1965.

7 Bowman, R. W. Instrument News *16* 2, 1965, Perkin Elmer Corporation.

8 Bowes, W. A., Drose, V. E. & Bruns, P. D. Amniocentesis and intrauterine fetal transfusion in erythroblastosis. Am J Obstet Gynec 93 822, 1965.

9 Bruns, J. V., Bowes, W. A. & Abbott, F. A. An improved rapid procedure for the determination of amniotic fluid bilirubin and its use in the prediction of the course of Rh-sensitized pregnancies. Am J Obstet Gynec *104* 80, 1969.

10 Brown, J. B., MacLeod, S. C., Macnaughtan, C., Smith, M. A. & Smyth, B. A rapid method for estimating oestrogens in urine using a semi-automatic extractor. J Endocrin *42* 5, 1968.

11. Cherry, S. H., Kochwa, S. & Rosenfield, R. E. Bilirubin–Protein ratio in amniotic fluid as an index of the severity of erythroblastosis fetalis. Obstet Gynec 26. 826, 1965.

12. Clayton, E. M., Walker, D. H. & Foster, E. B. The significance of hemepigments in amniotic fluid. Obstet Gynec *34* 641, 1969.

13. Efroymson, M. A. Multiple regression analysis. Mathematical methods for digital computers, part V (ed. A. Ralston & H. S. Wilf). J. Wiley & Sons, New York, 1964.

14 Fleming, E. A. & Woolf, A. J. A spectrophotometric method for the quantitative estimation of bilirubin in liquor amnii. Clin Chim Acta *12* 67, 1965.

15 Port, A. T. Prenatal intrusion into the amnion. Am J Obstet Gynec *110* 432, 1971.

16 Geyer, H. & Schedler, I. Enzyme in Fruchtwasser. Z Klin Chem Klin Biochem *8* 141, 1970.

17 Haemmerli, U. P. Jaundice during pregnancy. Acta Med Scand *179* Suppl. *444* 1966.

18. Halitsky, V. & Krumholz, B. A. Amniotic fluid analysis in erythroblastosis fetalis. III. The chloroform extract and its relationship to the log OD 450. Am J Obstet Gynec *106* 1209, 1970.

19 Ikonen, E. Jaundice in late pregnancy. Acta Obstet Gynec Scand *43* Suppl. 5, 1964.

20 Johansson, E. D. B. & Jonasson, L.-E. Progesterone levels in amniotic fluid and plasma from women. I. Levels during normal pregnancy. Acta Obstet Gynec Scand *50* 339, 1971.

21. Jonasson, L. E. & Johansson, E. D. B. Progesterone levels in amniotic fluid and plasma from women. II. Levels during pregnancies complicated by Rh-isoimmunization or hepatosis gravidarum. Acta Obstet Gynec Scand *50* 345, 1971.

22. Jonasson, L.-E. & Persson, B. H. Spectrophotometric screening of amniotic fluid in haemolytic disease and hepatosis gravidarum. Acta Obstet Gynec Scand *47* 300, 1968.

23 Jonasson, L.-E. Total protein content in amniotic fluid from normal pregnancies and in pregnancies complicated by Rh-isoimmunization. Acta Obstet Gynec Scand *51* 187, 1972.

24 Kuah, M. Y., Mirko, I. & Lack, H. Bilirubin estimation in amniotic fluid. Am J Obstet Gynec *106* 592, 1970.

25 Kubli, F. Die antenatale Diagnose des Rhesusbedingten Morbus Haemolyticus und die vorzeitige Beendigung der Schwangerschaft. Bibl Gynaec *38*, 30, 1966.

26 Lapan, B. & Friedman, M. M. Enzymes in the amniotic fluid and maternal serum. Am J Obstet Gynec *83* 1337, 1962.

27 Liley, A. W. Liquor amnii analysis in the management of the pregnancy complicated by rhesus sensitization. Am J Obstet Gynec *82* 1359, 1961.

28 — Errors in the assessment of hemolytic disease from amniotic fluid. Am J Obstet Gynec *86*. 485, 1963.

9 732493

29. Mandelbaum, B., La Croix, G. C. & Robinson A. R. Determination of fetal maturity by spectrophotometric analysis of amniotic fluid. Obstet Gynec 29 471 1967
30. Michaëlsson, M. Bilirubin determination in serum and urine. Scand J Clin Lab Invest, Suppl. 56, 1961.
31. Moltz, A., Pomerance, W & Blezenski, J J. Relationship of spectrophotometric analysis of amniotic fluid to the onset of spontaneous labor. Obstet Gynec 36 520, 1970.
32. Morris, E. D. Murray J & Ruthven, C. R. J. Liquor bilirubin levels in normal pregnancy: A basis for accurate prediction of hemolytic disease. Brit Med J 2 35_, 1967
33. Murray J. Norrie D. L. & Ruthven, C. R. J. Liquor bilirubin levels in normal pregnancy: A reassessment of early prediction of hemolytic disease. Brit Med J 4 387 1970.
34. McNay A., Oxley A. & Walker W. Comparison of chemical and spectrophotometric methods of estimating bilirubin in amniotic fluid. Am J Clin Path 50 122, 1968
35. Nilsson, B. A. Morbus haemolyticus neonatorum due to Rh-immunization. Acta Obstet Gynec Scand 44 Suppl. _ 69 1965
36. Ojala, A. Studies on bilirubin in amniotic fluid. Acta Obstet Gynec Scand 50 Suppl. 10: 30, 1971
37. Overstone J. A. & Connon, A. F. Optical density differencing: A new method for the direct measurement of bilirubin in liquor amnii. Clin Chim Acta 20 397 1968.
38. Parmley T & Miller E. Fetal maturity and amniotic fluid analysis. Am J Obstet Gynec, 105 354 1969
39. Queenan, J. T. & Goetschel, E. Views and reviews. Amniotic fluid analysis for erythroblastosis fetalis. Obstet Gynec 3 120, 1968.
40. Queenan, J. T., Gadow E. C., Bachner P & Kubarych, S. F. Amniotic fluid proteins in normal and Rh-sensitized pregnancies. Am J Obstet Gynec 108 406, 1970.
41. Stewart, A. G., Taylor W. C. & Beck, R. P. Amniotic fluid bilirubin levels and the Rh-isoimmunized mother. Am J Obstet Gynec 97 338 1967
42. Walker A. H. C. Liquor amnii studies in the prediction of hemolytic disease of the newborn. Brit Med J 2 376, 1957
43. Walker W. Landon, M J & Oxley A. Protein content of liquor amnii in prediction of severity of hemolytic disease of newborn. Brit Med J 1 665 1969
44. Watson, D. MacKay E. V. & Trevella, W. Amniotic fluid analysis and foetal erythroblastosis. Clin Chim Acta 1 500, 1965
45. White C. A., Doorenbos D. E. & Bradbury J T. Role of chemical and cytologic analysis of amniotic fluid in determination of fetal maturity. Am J Obstet Gynec 104 664, 1969
46. Willoughby H. W. Desjardins, P. D. Power R. M. H. & Lee T. K. Hyperbilirubinaemia in late pregnancy and its correlation with meconium staining. Am J Obstet Gynec 109 383, 1971

Submitted for publication June 1 1972

Lars Erik Jonasson
Department of Obstetrics and Gynaecology
University Hospital
S 750 14 Uppsala
Sweden

Acta Obstet Gynec Scand 52: 131–134 1973

CONTINUOUS RECORDING OF UTEROPLACENTAL BLOOD VOLUME IN THE HUMAN

T. H. Lippert, S. E. Cloeren, R. Fridrich and M. Hinselmann

From the Department of Obstetrics and Gynecology (Head: Prof. O. Käser) and the Department of Nuclear Medicine (Head: PD R. Fridrich), University of Basel, Switzerland

Abstract. A method for the continuous recording of uteroplacental blood volume has been described. A radioactive isotope (γ emitter) which is retained in the blood stream is injected into a cubital vein and the organ is surveyed externally using a γ detector. The activity measured is directly proportional to the blood volume surveyed by the detector. The method is satisfactory for organs with large blood volume compared to that of the tissues intervening between organ and detector. Therefore uteroplacental blood volume changes can be compared with those of other organs. Applications of the method in diagnosis and therapeutics have been discussed.

Due to its anatomical position investigation of the placental circulation has always presented difficulties. Techniques of measuring placental blood flow are laborious and often traumatic. Thus, in the present work, an attempt has been made to collate utero-placental blood volume with placental haemodynamics.

METHOD

8 μC of ^{113m}In-gelatin (a γ emitter) was injected into a cubital vein. This substance mixes uniformly throughout the blood, is bound to plasma proteins and does not leave the vascular system in appreciable amounts during the course of the investigation (7). Its relatively short physical half life (1.7 hours) is a slight disadvantage because decay which occurs during the course of the examination (30–60 min) is noticeable. However, correction for this can readily be made. Although more stable isotopes are available, this particular one was chosen for use in pregnant women due to its small radiation dose.

A multiple scintillation probe unit (Picker Nuclear Instruments) was used to measure continuously and record the γ activity with 3 detectors. After location of the placenta by ultrasound, one of the detectors was placed on the abdomen over the placental site. A second detector was placed over the heart and the third over placenta free uterine muscle or, in some cases, was used to show changes over another selected organ. The recording apparatus had only two channels, so the recordings illustrated are the ones of special significance to this paper namely the heart and placenta. The patient was lying in the supine position, turned slightly towards one side.

The radioactivity measured is directly proportional to the blood volume surveyed by the detector. This includes, in addition to the blood of the specific organ being examined, that of the skin and abdominal wall. However, since the latter two contribute only a small proportion to the total volume, they may usually be neglected. After 30 min the measured activity is about 80% of the original value, so that changes in blood volume are obvious without decay calculations and the observations are adequate for relative work.

RESULTS

Fig. 1 shows typical curves of uteroplacental and heart blood volumes. The patient was a 26-year-old para II, in the 27th week of pregnancy with a normal case history. The following observations were made from the tracings:

1. *Build-up time—i.e. time from injection into* the cubital vein until the maximum number of counts/min is reached.

2. Equilibrium time—i.e. time taken for the ^{113m}In-gelatin and normal plasma to be thoroughly and evenly distributed throughout the entire blood volume. The state of equilibrium is indicated by a plateau.

3. Fluctuations due to changes in volume occurring after equilibrium has been reached. These fluctuations were observed for 20–30 min. Usually they are small but at or near term they become larger due to spontaneous contractions of the uterus.

29 Mandelbaum, B., La Croix, G C. & Robinson A. R. Determination of fetal maturity by spectrophotometric analysis of amniotic fluid. Obstet Gynec *29* 471 1967
30. Michaëlsson, M. Bilirubin determination in serum and urine Scand J Clin Lab Invest, Suppl. *56*, 1961
31 Moltz, A., Pomerance W & Biezenski, J J Relationship of spectrophotometric analysis of amniotic fluid to the onset of spontaneous labor Obstet Gynec *36* 520, 1970.
32. Morris, E. D Murray J & Ruthven, C. R. J Liquor bilirubin levels in normal pregnancy: A basis for accurate prediction of hemolytic disease Brit Med J *2* 352, 1967
33 Murray J Norrie, D L. & Ruthven, C. R. J Liquor bilirubin levels in normal pregnancy: A reassessment of early prediction of hemolytic disease. Brit Med J *4* 387 1970.
34 McNay A., Oxley A. & Walker W Comparison of chemical and spectrophotometric methods of estimating bilirubin in amniotic fluid. Am J Clin Path *50* 122, 1968
35 Nilsson, B. A. Morbus haemolyticus neonatorum due to Rh-immunization. Acta Obstet Gynec Scand *44* Suppl. _ 69 1965
36. Ojala, A. Studies on bilirubin in amniotic fluid. Acta Obstet Gynec Scand *50* Suppl. 10: 30 1971
37 Overstone, J A. & Connon, A. F Optical density differencing: A new method for the direct measurement of bilirubin in liquor amnii. Clin Chim Acta *20* 397 1968.
38. Parmley T & Miller E. Fetal maturity and amniotic fluid analysis. Am J Obstet Gynec, *105* 354 1969
39 Queenan, J T & Goetschel, E. Views and reviews. Amniotic fluid analysis for erythroblastosis fetalis. Obstet Gynec *32* 120 1968.
40 Queenan, J T Gadow E. C., Bachner P & Kubarych, S. F Amniotic fluid proteins in normal and Rh-sensitized pregnancies. Am J Obstet Gynec *108*, 406, 1970
41 Stewart, A. G., Taylor W C. & Beck, R. P Amniotic fluid bilirubin levels and the Rh-isoimmunized mother Am J Obstet Gynec *97* 338 1967
4_ Walker A. H. C. Liquor amnii studies in the prediction of hemolytic disease of the newborn. Brit Med J *2* 376, 1957
43. Walker W Landon, M. J & Oxley A. Protein content of liquor amnii in prediction of severity of hemolytic disease of newborn. Brit Med J *1* 605 1969
44 Watson, D., Mackay E V & Trevella, W Amniotic fluid analysis and foetal erythroblastosis. Clin Chim Acta *12* 500, 1965
45 White C. A., Doorenbos, D. E. & Bradbury J. T Role of chemical and cytologic analysis of amniotic fluid in determination of fetal maturity Am J Obstet Gynec *104* 664 1969
46. Willoughby H. W Desjardins, P D., Power R. M. H & Lee, T K. Hyperbilirubinaemia in late pregnancy and its correlation with meconium staining. Am J Obstet Gynec *109* 383, 1971

Submitted for publication June 1 1972

Lars Erik Jonasson
Department of Obstetrics and Gynaecology
University Hospital
S 750 14 Uppsala
S eden

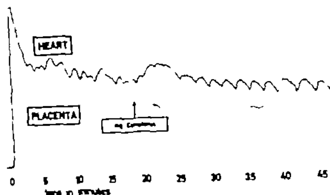


Fig. 3. Blood volume tracings taken over the heart and placenta of 19-year-old para 0 in the 40th week of normal pregnancy illustrating rapid onset of action of an intravenous injection of 75 mg Complamin. The ordinate shows activity in counts/min and the abscissa the time in min.

DISCUSSION

Interpretation of the tracings has been made according to their two distinct phases. The first functional part of the curve has been considered until equilibrium was reached. It gives, therefore, measure of build-up time and of equilibrium time, both measurements relating to blood flow. The build-up time gives a measure of the speed of transport of activity from the cubital vein to the organ surveyed. On the other hand, equilibrium time is informative not only on the circulation of the organ but also on the general body circulation, the activity being then uniformly distributed throughout the entire blood volume. In the present investigations, the difference between build-up time and equilibrium time was specially marked in the heart tracings, was usually less obvious in the placenta, while in the uterine muscle it was often inconspicuous. Using radio-iodinated (^{131}I) human serum albumin (RIHSA) as tracer, Smith (6) calculated the turnover rate of placental blood in cases of discrepancy between the equilibrium times of heart and placenta. However, such calculations are open to criticism since it is often difficult to define when equilibrium has been reached, due to fluctuations. In our experience, the equilibrium times of heart and placenta were very often similar. Thus, in the work reported here, the build-up and equilibrium times, as indications of flow rate, have been compared only relatively.

The second functional part of the tracings indicates blood volume changes. Having reached equilibrium, the numbers of counts give relative values for the amount of blood in the organ surveyed. Generally there was more blood in the heart than in the placenta, while the non-placental uterine muscle had still less. In measurements over the placenta, there is, in addition to the placental blood, the blood in the uterine muscle to which the placenta is attached. To obtain a measure of placental blood volume alone, Scheffs et al. (5) subtracted the uterine muscle blood volume from the total uteroplacental volume. However, as Jansson (4) has shown, uterine muscle connected to placenta is much better perfused than placenta-free uterine muscle. Thus, applying Fourier's analysis would not appear to give accurate measurements of placental blood volume. In the present work no attempt has been made to assess blood volume in the placenta alone, as tracings for the placenta include the blood of the uterine muscle. The blood volume parameter so measured provides useful informa-



Fig. Blood volume tracings taken over the heart and placenta in 39-year-old para II in the 40th week of pregnancy with signs of fetal distress. The tracings illustrate delayed onset of action following an intravenous injection of 75 mg Complamin. The ordinate shows activity in counts/min and the abscissa the time in min.

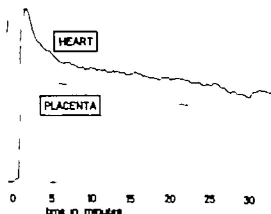


Fig 1 Blood volume tracings t ken over the heart and placenta in a 26-year-old patient, para II in the 27th week of normal pregnancy The ordinate show activity in count /min and the abscissa, the time in min.

Fig. 2 shows tracings from a 33-year-old para I in the 39th week of pregnancy She was admitted to hospital already experiencing uterine contrac tions and these continued during recording of the curves illustrated. The uterine activity was reflected in the blood volume changes seen in the tracings. Typically a decrease in uteroplacental blood volume is associated with a corresponding increase in blood volume of the heart and vice versa

Having obtained equilibrium and observed the spontaneous fluctuations, the actions of drugs on uteroplacental blood volume can be examined using this method Xanthinol nicotinate (Compla min) is used in vascular disease in order to im prove circulation It has been suggested that placental circulation and function might also be improved by its use (1) and therefore it was included in our investigations. Fig 3 shows the action of an intravenous injection of 75 mg Complamin in a healthy 19-year-old para 0 in the 40th week of pregnancy There was an im mediate onset of action the uteroplacental blood volume showing a transitory decrease A corresponding reciprocal action of the heart blood volume was observed. However a sustained im provement in placental circulation was not shown These actions were observed repeatedly and seem to be typical for Complamin in a normal preg nancy showing good reactivity of the vascular system.

Fig 4 shows a different effect of Complamin obtained in a 39-year-old para II in the 40th week of pregnancy Fluctuations were observed for about 20 min during which a spontaneous uterine contraction occurred. Then 75 mg Complamin were injected intravenously and only after 4 min was a delayed action observed. The uteroplacental blood volume decreased and remained low No corresponding change was observed in heart blood volume This reaction may be due to reduced reactivity of the vessels and to pathological conditions prevailing. In this case the build-up time for the uteroplacental blood was about 4 min, compared with 2 min in the previous case (Fig 3) Also the fluctuations of both placental and heart recordings were much less marked. These observations seem to indicate a less efficient circulation of blood in the placenta and poorer reactivity of the vessels.

This patient (Fig 4) in contrast to the former had a complicated case history She had previously had one intrauterine fetal death at term and one viable child delivered by caesarean section because of breech presentation combined with fetal distress. In the present pregnancy she was admitted to hospital for observation in the 37th week because of suspected fetal growth retardation. From this time on daily fetal heart rate monitoring showed signs of placental insufficiency as seen by reduced baseline fluctuations. When late decelerations of the fetal heart rate were observed (again a breech presentation) a caesarean section was performed. The outcome was good, the child having an Apgar score of 9 10 10



F Blood olume tracings taken over the heart and placenta in a 33-yea -old para I in the 39th week of normal pregnancy demonstrating the ffects of uterine contractions The rdinate show activity in counts min and the abscissa the time in min.

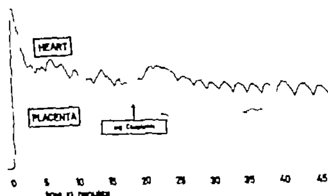


Fig. 3. Blood volume tracings taken over the heart and placenta of 19-year-old para 0 in the 40th week of normal pregnancy illustrating rapid onset of action of an intravenous injection of 75 mg Complamin. The ordinate shows activity in counts/sec and the abscissa the time in min.

DISCUSSION

Interpretation of the tracings has been made according to their two distinct phases. The first functional part of the curve has been considered until equilibrium was reached. It gives, therefore, a measure of build-up time and of equilibrium time, both measurements relating to blood flow. The build-up time gives a measure of the speed of transport of activity from the cubital vein to the organ surveyed. On the other hand, equilibrium time is informative not only on the circulation of the organ but also on the general body circulation, the activity being then uniformly distributed throughout the entire blood volume. In the present investigations, the difference between build-up time and equilibrium time was specially marked in the heart tracings, was usually less obvious in the placenta, while in the uterine muscle it was often inconspicuous. Using radioiodinated (^{131}I) human serum albumin (RIHSA) as tracer Smith (6) calculated the turnover rate of placental blood in cases of discrepancy between the equilibrium times of heart and placenta. However such calculations are open to criticism since it is often difficult to define when equilibrium has been reached, due to fluctuations. In our experience the equilibrium times of heart and placenta were very often similar. Thus, in the work reported here, the build-up and equilibrium times, as indications of flow rate, have been compared only relatively.

The second functional part of the tracings indicates blood volume changes. Having reached equilibrium, the numbers of counts give relative values for the amount of blood in the organ surveyed. Generally there was more blood in the heart than in the placenta while the non-placental uterine muscle had still less. In measurements over the placenta, there is, in addition to the placental blood, the blood in the uterine muscle to which the placenta is attached. To obtain a measure of placental blood volume alone Scheffs et al. (5) subtracted the uterine muscle blood volume from the total uteroplacental volume. However as Jammon (4) has shown, uterine muscle connected to placenta is much better perfused than placenta free uterine muscle. Thus, applying Fourier analysis would not appear to give accurate measurements of placental blood volume. In the present work no attempt has been made to assess blood volume in the placenta alone so tracings for the placenta include the blood of the uterine muscle. The blood volume parameter so measured provides useful informa-



Fig. 4. Blood volume tracings taken over the heart and placenta in 39-year-old para II in the 40th week of pregnancy with signs of fetal distress. The tracings illustrate delayed onset of action following an intravenous injection of 75 mg Complamin. The ordinate shows activity in counts/sec and the abscissa the time in min.

tion on hemodynamics without giving actual measurements of blood flow

In such volume measurements, the observation of fluctuation is of special interest. It is well-known that the volume of an organ undergoes spontaneous and rhythmic variations. These can be attributed to hemometakinesia the so-called borrowing lending phenomenon already described by DeBakey et al. (7) However the placenta is an exception to other organs since volume changes are contributed to not only by vasoconstrictor or dilator influences on the vessels but also by uterine muscle activity Intraamniotic pressure recordings taken concurrently can indicate the role of uterine muscle activity in causing placental blood volume changes. Pulse and respiratory deflections, as described in plethysmography measurements in peripheral parts (e.g. fingers) are not registered using this method but only larger blood volume changes.

The described method is specially applicable to drug action studies. The drug's effect can be observed simultaneously on different organs so that changes in blood distribution in various parts of the body may be seen. In this way the clinical value of a drug can be better assessed. The action of Complamin on the uteroplacental blood volume was examined for its possible use as a diagnostic test. A delayed onset of action with decreased blood volume in the placenta may well be a sign of pathological conditions. Other drugs are at present under investigation

The technique here described is simple, atraumatic and presents no danger to mother or fetus. The radiation dose of In^{113m} is small for the fetus (about 5 mrad) and is only about one quarter of that used in placentography where In^{113m} has already established use (3) Compared with RIHSA which was used by Smith (6) and by Schells et al. (5) the radiation dose is smaller and there is no danger of free radioactive iodine passing into the fetal circulation.

The method may be applied as a routine test for the diagnosis of inadequate placental circulation. Meanwhile, the evaluation of large numbers of normal and pathological cases is required before a valid assessment can be made.

REFERENCES

1 Bergstein, N A. M & Kessel v H. J J M. Xanthinol nicotinate and placental function. Lancet ... 111, 1968

2. DeBakey M. E., Bruch, G Ray T & Ochsner A. The "borrowing-lending" hemodynamic phenomenon (Hemometakinesia) and its therapeutic application in peripheral vascular disturbances. Ann Surg 1?6, 1?0, 1947

3 Fridrich, R., Meudt, R. & Rippmann, E. T Nuklearmedizinische Probleme bei der Erfassung der Placentafunktion. *In* Die Spätgestose p 36. Schwabe Verlag Basel, 1969

4 Jonsson, J ¹³³Xenon clearance in the myometrium of pregnant and non-pregnant women. Acta Obstet Gynec Scand *48* 302, 1969

5. Schells, J Vasicka, A., Li, C., Solomon, N & Siler, W Uterine blood flow during labor Obstet Gynec 3? 15 1971

6. Smith, R. W Cardiovascular alterations in toxemia. Amer J Obstet Gynec *107* 979 1970.

7 Stern, H. S., Goodwin, D A., Scheffel, U Wagner, H. N & Kramer H H. In ¹¹³m for blood pool and brain scanning. Nucleonics 2? 6?, 1967

Submitted for publication June 19 1972

T H Lippert
Department of Obstetrics and Gynecology
University Hospital
Basel
Switzerland

Acta Obstet Gynec Scand 52: 135–140, 1973

METABOLISM OF GLUCOSE IN THE HUMAN ENDOMETRIUM WITH SPECIAL REFERENCE TO FERTILITY AND CONTRACEPTION

Heinrich Hackl

From the Department of Obstetrics and Gynaecology (Head, Professor S. Kullander), University of Lund, Malmö, Sweden

Abstract. On the basis of numerous biopsy specimens of the endometrium, in which substrates and enzymes concerned in the metabolism of glucose were examined in vitro, the following observations were made. Respiration and redox capacity as well as endogenous and exogenous glycolysis of the endometrium are increased maximally during the secretory phase. In this phase also the absolute Pasteur effect is increased to a maximum, while the control of glycolysis by the substrate is decreased. In addition, a Crabtree-effect appears at the same time. This suggests that the endometrium in the implantation phase endeavours to deliver constant amounts of ATP and to maintain a stable physico-chemical environment. While from a gynaecological point of view various endometrium-associated disorders cause no notable differences in metabolism, the uptake of glucose, or respiration, cytochrome oxidase and succinate dehydrogenase activity in the secretory endometrium decrease with advancing age and thereby presumably diminish the fertility of the elderly woman. The use of oral contraceptives reduces the glucose metabolism from endogenous and from exogenous substrates in the endometrium, despite increasing synthesis of glycogen during the cycle. The reduction of metabolism occurs to such an extent that it must be expected that a contraceptive effect in the endometrium takes place.

Various investigations concerning glucose metabolism in the human endometrium were published between 1929 and 1967 but the studies were on small series of cases and the findings are therefore difficult to interpret with certainty (1, 8, 9, 11, 16, 19, 20, 21 ... 29). Therefore it was decided to study various substrates of glucose metabolism in the endometrium in a larger series of patients, with special reference to certain gynaecological aspects. Such data could be used also for comparisons between women using and not using oral contraceptives.

MATERIAL AND METHODS

Biopsy material was obtained from about 1 500 patients undergoing curettage for various reasons at the University Department of Obstetrics and Gynaecology, Malmö. Histologically normal endometrium was subjected to differentiated in vitro-studies including the following: the utilisation of glucose in the medium (4), the O_2-uptake by ordinary Warburg technique, the formation of CO_2 from glucose-C-6 and from glucose-C-1 by combined Warburg isotope technique, the formation of lactate (17) and the glycogen content (26). The investigations were also extended to include quantitative determination of lactate dehydrogenase activity determined in an Autoanalyzer (28) and succinate dehydrogenase and cytochrome oxidase activity both determined manometrically (30). The preparation of the samples was described in detail by Hackl (1970).

THE MENSTRUAL CYCLE

The utilisation of glucose added to the medium did not vary in any particular way with the various phases of the cycle (13). This is in agreement with the findings of Hagerman & Villée 1953 (16). The consumption of glucose tended to be reduced around the middle of the cycle and to rise slightly towards the end of the cycle. In four series the formation of lactate clearly increased from the proliferative phase (*p*-phase) to the secretoric phase (*s*-phase). In most series this increase was successive and a metabolic peak was observed in the aerobic and anaerobic glycolysis between the 19th and the 25th day of the cycle.

Addition of glucose to the medium (up to 10 mM) stimulated the formation of lactate in the *p*-phase. In the *s*-phase up to 5.5 mM glucose concentration, this stimulation was only moderate and up to 10 mM it was no longer demonstrable with certainty. This suggests that glycolysis is

tion on hemodynamics without giving actual measurements of blood flow

In such volume measurements, the observation of fluctuation is of special interest. It is well-known that the volume of an organ undergoes spontaneous and rhythmic variations. These can be attributed to hemometakinesia, the so-called borrowing lending phenomenon already described by DeBakey et al. (2) However the placenta is an exception to other organs since volume changes are contributed to not only by vasoconstrictor or dilator influences on the vessels but also by uterine muscle activity Intraamniotic pressure recordings taken concurrently can indicate the role of uterine muscle activity in causing placental blood volume changes. Pulse and respiratory deflections, as described in plethysmography measurements in peripheral parts (e.g. fingers) are not registered using this method but only larger blood volume changes.

The described method is specially applicable to drug action studies. The drug s effect can be observed simultaneously on different organs so that changes in blood distribution in various parts of the body may be seen In this way the clinical value of a drug can be better assessed. The action of Complamin on the uteroplacental blood volume was examined for its possible use as a diagnostic test. A delayed onset of action with decreased blood volume in the placenta may well be a sign of pathological conditions. Other drugs are at present under investigation.

The technique here described is simple atraumatic and presents no danger to mother or fetus. The radiation dose of In^{113m} is small for the fetus (about 5 mrad) and is only about one quarter of that used in placentography where In^{113m} has already established use (3). Compared with RIHSA which was used by Smith (6) and by Scheffs et al. (5) the radiation dose is smaller and there is no danger of free radioactive Iodine passing into the fetal circulation.

The method may be applied as a routine test for the diagnosis of inadequate placental circulation. Meanwhile, the evaluation of large numbers of normal and pathological cases is required before a valid assessment can be made.

REFERENCES

1 Bergstem, N. A. M. & Kessel v. H. J. J. M. Methyl nicotinate and placental function. Lancet 111, 1968

2. DeBakey M. E., Burch, G., Ray T & Ochsner, A. The "borrowing-lending" hemodynamic phenomenon (Hemometakinesia) and its therapeutic application in peripheral vascular disturbances. Ann Surg 1?5 ?? 1947

3. Fridrich, R., Mendt, R. & Rippmann, E. T Nuklearmedizinische Probleme bei der Erfassung der Plazentafunktion. *In* Die Spätgestose p. ?86. Schwabe Verlag Basel, 1969

4 Jansson, J. [133]Xenon clearance in the myometrium of pregnant and non-pregnant women. Acta Obstet Gynec Scand 48 302, 1969

5 Scheffs, J Vasicka, A., Li, C., Solomon, N. & Stet, W Uterine blood flow during labor Obstet Gynec 38 15 1971

6. Smith, R. W Cardiovascular alterations in toxemia. Amer J Obstet Gynec *107* 979 1970.

7 Stern, H. S., Goodwin, D A., Scheffel, U Wagner, H. N. & Kramer H. H. In [113m] for blood-pool and brain scanning Nucleonics 25 62, 1967

Submitted for publication June 19 197

T H Lippert
Department of Obstetrics and Gynecology
University Hospital
Basel
Switzerland

Acta Obstet Gynec Scand 52: 135–140 1973

METABOLISM OF GLUCOSE IN THE HUMAN ENDOMETRIUM WITH SPECIAL REFERENCE TO FERTILITY AND CONTRACEPTION

Heinrich Hackl

From the Department of Obstetrics and Gynaecology (Head: Professor S. Kullander), University of Lund, Malmö, Sweden

Abstract. On the basis of numerous biopsy specimens of the endometrium, in which substrates and enzymes concerned in the metabolism of glucose were examined *in vitro*, the following observations were made. Respiration and redox capacity as well as endogenous and exogenous glycolysis of the endometrium are increased maximally during the secretory phase. In this phase also the absolute Pasteur effect is increased to a maximum, while the control of glycolysis by the substrate is decreased. In addition, a Crabtree-effect appears at the same time. This suggests that the endometrium in the implantation phase endeavours to deliver constant amounts of ATP and to maintain a stable physico-chemical environment. While from a gynaecological point of view various endometrium-associated disorders cause no notable differences in metabolism, the uptake of glucose or respiration, cytochrome oxidase and succinate dehydrogenase activity in the secretory endometrium decrease with advancing age and thereby presumably diminish the fertility of the elderly woman. The use of oral contraceptives reduces the glucose metabolism from endogenous and from exogenous substrates in the endometrium, despite increasing synthesis of glycogen during the cycle. The reduction of metabolism occurs to such an extent that it must be expected that a contraceptive effect in the endometrium takes place.

Various investigations concerning glucose metabolism in the human endometrium were published between 1929 and 1967 but the studies were on small series of cases and the findings are therefore difficult to interpret with certainty (1 8, 9 11 16, 19 20 21 22, 29). Therefore it was decided to study various substrates of glucose metabolism in the endometrium in a larger series of patients, with special reference to certain gynaecological aspects. Such data could be used also for comparisons between women using and not using oral contraceptives.

MATERIAL AND METHODS

Biopsy material was obtained from about 1 500 patients undergoing curettage for various reasons at the University Department of *Obstetrics and Gynaecology* Malmö. Histologically normal endometrium was subjected to differentiated in vitro-studies including the following: the utilisation of glucose in the medium (4), the O_2-uptake by ordinary Warburg technique, the formation of CO_2 from glucose-C-6 and *from glucose-C-1 by combined* Warburg isotope technique, the formation of lactate (17) and the glycogen content (26). The investigations were also extended to include quantitative determination of lactate dehydrogenase activity determined in an Auto-analyzer (28) and succinate dehydrogenase *and cytochrome* oxidase activity both determined manometrically (30). The preparation of the samples is described in detail by Hackl (1970).

THE MENSTRUAL CYCLE

The utilisation of glucose added to the medium did not vary in any particular way with the various phases of the cycle (13). This is in agreement with the findings of *Hagerman & Villée* 1953 (16). The consumption of glucose tended to be reduced around the middle of the cycle and to rise slightly towards the end of the cycle. In four series *the formation of lactate clearly increased* from the proliferative phase (*p*-phase) to the secretoric phase (*s*-phase). In most series this increase was successive and a metabolic peak was observed in the aerobic and anaerobic glycolysis between the 19th and the 25th day of the cycle.

Addition of glucose to the medium (up to 10 mM) stimulated the formation of lactate in the *p*-phase. In the *s*-phase, up to 5.5 mM glucose concentration, this stimulation was only moderate and up to 10 mM it was no longer demonstrable with certainty. This suggests that glycolysis is

tion on hemodynamics without giving actual measurements of blood flow

In such volume measurements, the observation of fluctuation is of special interest. It is well known that the volume of an organ undergoes spontaneous and rhythmic variations. These can be attributed to hemometakinesia the so-called borrowing-lending phenomenon already described by DeBakey et al. (2) However the placenta is an exception to other organs since volume changes are contributed to not only by vasoconstrictor or dilator influences on the vessels but also by uterine muscle activity Intraamniotic pressure recordings taken concurrently can indicate the role of uterine muscle activity in causing placental blood volume changes. Pulse and respiratory deflections, as described in plethysmography measurements in peripheral parts (e.g. fingers) are not registered using this method but only larger blood volume changes.

The described method is specially applicable to drug action studies. The drug s effect can be observed simultaneously on different organs so that changes in blood distribution in various parts of the body may be seen In this way the clinical value of a drug can be better assessed. The action of Complamin on the uteroplacental blood volume was examined for its possible use as a diagnostic test. A delayed onset of action with decreased blood volume in the placenta may well be a sign of pathological conditions. Other drugs are at present under investigation

The technique here described is simple atraumatic and presents no danger to mother or fetus. The radiation dose of In^{113m} is small for the fetus (about 5 mrad) and is only about one quarter of that used in placentography where In^{113m} has already established use (3) Compared with RIHSA which was used by Smith (6) and by Schefls et al. (5) the radiation dose is smaller and there is no danger of free radioactive Iodine passing into the fetal circulation.

The method may be applied as a routine test for the diagnosis of inadequate placental circulation Meanwhile the evaluation of large numbers of normal and pathological cases is required before a valid assessment can be made.

REFERENCES

1 Bergstein, N. A. M. & Kessel v H. J. J. M Xanthinol nicotinate and placental function. Lancet 111, 1968
2 DeBakey M. E., Bruch, G Ray T & Ochsner A. The "borrowing-lending" hemodynamic phenomenon (Hemometakinesia) and its therapeutic application in peripheral vascular disturbances. Ann Surg 126 850, 1947
3 Fridrich, R., Mendt, R. & Rippmann, E. T Nuklearmedizinische Probleme bei der Erfassung der Placentafunktion. I Die Spätgestose p. 286. Schwabe Verlag, Basel, 1969
4 Jansson, I., 133Xenon clearance in the myometrium of pregnant and non-pregnant women. Acta Obstet Gynec Scand 48, 302, 1969
5 Schefls, J Vasicka, A., Li, C., Solomon, N. & Siler W Uterine blood flow during labor Obstet Gynec 38 15 1971
6 Smith, R. W Cardiovascular alterations in toxemia. Amer J Obstet Gynec 107 979 1970.
7 Stern, H S., Goodwin, D A., Scheffel, U Wagner H N & Kramer H. H., In 113m for blood pool and brain scanning. Nucleonics 5 62, 1967

Submitted for publication June 19 1972

T H. Lippert
Department of Obstetrics and Gynecology
University Hospital
Basel
Switzerland

minations showed the variation of the glycogen content in the s-phase to vary with anaerobic glycolysis. The same study with endogenous respiration showed, if anything, a negative correlation which may probably be explained by the known competition between glycolysis and respiration.

With the aid of various large doses of progesterone and the administration of oestradiol to patients in the p-phase the synthesis of glycogen in the endometrium was studied experimentally (15). It was found that initial glycogen synthesis does not vary with the dose of progesterone given. The synthesis increased through the p-phase in which the progesterone was given but was independent of dosage. This thus suggests the striking effect of oestrogen priming resp. of the oestrogen level. Application of 25 mg progesterone which judging from the excretion of pregnandiol, corresponds to the physiological progesterone level in the secretory phase, did not increase the O_2-uptake, but it did increase the formation of lactate in the endometrium from the endogenous substrates as well as in the presence of glucose in the medium.

Unlike progesterone, oestradiol did not increase endometrial glycogen synthesis. As suggested by Hughes et al. 1969 (18) and Vasquez et al. 1968 (31) the synthesis of glycogen is probably initiated by progesterone but the rate of synthesis is controlled by oestrogens.

According to Hughes et al. 1969 (18), progesterone stimulates phosphorylase activity and, judging from the present results, also the formation of lactate from glycogen. Bearing in mind the relatively constant glycogen level during the secretory phase (13) one might assume that the maximum secretion of progesterone during the implantation phase has the function of breaking down the glycogen faster while at the same time the oestrogens have the function of synthesizing glycogen in increased amounts. In other words, this would mean a larger passage of glycogen during the proper secretory phase than during the presecretory phase and this would favour endogenous glycolysis at the time for implantation.

VARIOUS CATEGORIES OF ENDOMETRIUM

Routine endometrial specimens were compared with those from women with menstrual disorders and women with myomata (13). Such an investigation was considered a desirable preliminary to a planned later comparison between the endometrium from women using and not using oral contraceptives.

No difference were found between the glucose metabolism and lactate formation in the various categories of endometrium. On the other hand, differences were found in the O_2-uptake and glucose-C-6-oxidation in that the two pathological groups showed no clear difference or no difference at all between the two cyclic phases. Such a finding could be a manifestation of disturbed oxidative breakdown of glucose owing to disturbed vascularisation, possibly also as a consequence of local differences in metabolism and compensation mechanisms in an inadequately developed functional layer. Judging from the observations of Schmidt-Mathiesen 1965 (27) one would have expected an abnormal glycogen content in patients with bleeding anomalies. But this was not the case, possibly because the bleeding anomaly had ceased at the time of curettage. Evidence of changed glycogen content in various gynaecological diseases may therefore be difficult to obtain because glycogen can probably be converted with the mucopolysaccharides.

GLUCOSE METABOLISM AND AGE

Glucose metabolism was studied for variation with age on the basis of three groups (≤35, ≥36–45, ≥46 years) and continuous age curves (13). It was found that the glucose consumption in the secretory endometrium in women up to 46 years was decreased. But this was not the case with lactate formation, which under anaerobic conditions tended, if anything, to increase. With increasing age the O_2-uptake successively decreased between the 19th and the 25th day of the cycle. This was also true for the two oxreductases during the entire s-phase in which the cytochrome oxidase proved the better indicator for age. This decrease with age of the three parameters of oxidative metabolism may indicate that the formation of ATP in the aging oxidation medium decreases. It must be borne in mind that a given number of glucose molecules in the aerobic pathway yields about 20 times more ATP than on anaerobic glycolysis.

Neither in the p-phase nor in the s-phase did

more substrate-linked in the proliferative than in the secretory endometrium.

Judging from the direct correlation between the glucose utilisation and the formation of lactate under aerobic conditions about 1 2 mol of lactic acid forms from 1 mol of glucose consumed and under anaerobic conditions, about 1 6 mol these figures remained fairly constant throughout the cycle As expected, not only the formation of lactate but also glucose utilisation was higher under anaerobic than under aerobic conditions. In physiological environments, i.e. 5.5 mM in vitro about 20–30% of added glucose will be converted to lactic acid in the human endometrium under optimal conditions, i.e. under anaerobic conditions in the *s*-phase the conversion will be 50% Since 2 mol of lactic acid can be formed from 1 mol of glucose the degree of glycolysis in the human endometrium appears fairly low However under aerobic conditions, the formation of lactate from endogenous substrates in the *s*-phase was strikingly high

The curves for the formation of lactate and LDH activity through the menstrual cycle were similar and they varied equally and significantly with the phases of the cycle. This suggests that in addition to the observed substrate linkage lactate dehydrogenase also has a governing influence on glycolysis.

As in the formation of lactate the O_2-uptake from the endogenous substrate of the endometrium increased from the *p*- to the *s*-phase. However when additional amounts of glucose were *added to the medium the difference* between the phases of the cycle became smaller as a consequence of respiratory increase in the *p*-phase and respiratory inhibition in the *s* phase. In view of the relatively high aerobic glycolysis the respiratory inhibition in the *s* phase was interpreted as a Crabtree effect of the endometrium (3 7)

In various glucose *concentrations in the medium*, the oxidation of glucose-C-6 which is a measure of the turnover through the citric acid cycle, varied inversely with the rest of the metabolic features investigated. This oxidation in the *s* phase was thus lower which might have been due either to a high endogenous glucose metabolism (large glycogen pool) or to a high lipoid or protein *metabolism in this phase*. The absolute values found for glucose-C-6-oxidation thus allowed no conclusion about the true order of the glucose breakdown through the citric acid cycle.

In another series of biopsies glucose-C-6-oxidation and glucose-C 1-oxidation were compared (1-C-CO_2 minus 6-C-CO) (14) Since only 1-C labelled glucose can be oxidased in pentose phosphate shunt it was possible in this way to assess the absolute size of the shunt in the endometrium (2) The experiments were performed with 0 5 and 5 5 mM glucose in the medium. It was found that the shunt corresponding to the highest mitotic activity was highest during late proliferation At the same time however the variation of the values measured was also widest. The comparison of the values found for the two glucose concentrations of the medium revealed that the shunt was substrate linked between these concentrations. Compared with the total glucose metabolism of the endometrium that of the shunt was very small These results of the maximum substrate breakdown by the pentose phosphate shunt in the late *p*-phase are incompatible with the enzyme activities of the shunt found by Hughes et al. (19) as well as by Luh & Brandau (23) These appear to be too high to limit the shunt

In order to elucidate the redox capacity of the endometrium the two oxireductases, succinate dehydrogenase and cytochrome oxidase, were determined in a certain number of cases. In previous animal experiments it had been shown that these two enzymes could be influenced by *steroid hormones (5 10 12). It was found* that both enzyme activities—like the O_2-uptake—increased from the *p*- to the *s*-phase

In secretory endometrium the quantitatively determined glycogen content is six times as high as in the *p*-phase (13) Determination of the breakdown of the glycogen in vitro showed that this increases successively during the cycle to *reach a metabolic peak between the 19th* and the *25th day* with a simultaneous decrease of the relative breakdown of glycogen i e the relation between the amount of glycogen present and that broken down This suggests a dual function of the glycogen in the secretory endometrium It contributes to an increase and thereby stabilisation of metabolism of the endogenous substrate and also acts as an energy reserve for development of a placenta if necessary Paired deter

lnations showed the variation of the glycogen in the s-phase to vary with anaerobic sis. The same study with endogenous lration showed, if anything, a negative correla ion which may probably be explained by the n competition between glycolysis and respira-

With the aid of various large doses of pro- and the administration of oestradiol to in the p-phase, the synthesis of glycogen the endometrium was studied experimentally (15). It was found that initial glycogen synthesis not vary with the dose of progesterone given. The synthesis increased through the p-phase in which the progesterone was given, but was independent of dosage. This thus suggests the strik effect of oestrogen priming resp. of the level. Application of 25 mg progeste which judging from the excretion of pregnandiol, corresponds to the physiological progesterone level in the secretory phase, did not increase the O_2-uptake, but it did increase the ormation of lactate in the endometrium from the genous substrates as well as in the presence of glucose in the medium.

Unlike progesterone, oestradiol did not increase metrial glycogen sy thesis. As suggested by et al. 1969 (18) and Vasquez et al. 1968 (31) the synthesis of glycogen is probably initiated progesterone, but the rate of synthesis is controlled by oestrogens.

According to Hughes et al. 1969 (18), pro-one stim lates phosphorylase activity and, judging from the present results, also the formation of lactate from glycogen. Bearing in mind he relatively constant glycogen level during the tory phase (13), one might assume that the imum secretion of progesterone during the implantation phase has the function of breaking n the glycogen faster while at the same time he oestrogen ha e the function of synthesizing ycogen in increased amounts. In other words, ould mean larger passage of glycogen ing the proper secretory phase than during the secretory phase and this would favour endo- ial glycolysis t the time for implantation.

VARIOUS CATEGORIES OF ENDOMETRIUM

The endometrial specimens were compared th those from women with menstrual disorders and women with myomata (13). Such an investigation was considered a desirable preliminary to a planned later comparison between the endometrium from women using and not using oral contraceptives.

No difference were found between the glucose metabolism and lactate formation in the various categories of endometrium. On the other hand, differences were found in the O_2-uptake and glucose-C-6-oxidation in that the two pathological groups showed no clear difference or no dif ference at all between the two cyclic phases. Such a finding could be a manifestation of disturbed oxidative breakdown of glucose owing to disturbed vascularisation, possibly also as a consequence of local differences in metabolism and compensation mechanisms in an inadequately developed functional layer. Judging from the observations of Schmidt-Mathiesen 1965 (27) one would have expected an abnormal glycogen con tent in patients with bleeding anomalies. But this was not the case possibly because the bleeding anomaly had ceased at the time of curettage. Evidence of a changed glycogen content in various gynaecological diseases may therefore be difficult to obtain because glycogen can probably be converted with the mucopolysaccharides.

GLUCOSE METABOLISM AND AGE

Glucose metabolism was studied for variation with age on the basis of three groups (< 35 > 36–45 > 46 years) and continuous age curves (13). It was found that the glucose consumption in the secretory endometrium in women up to 46 years was decreased. But this was not the case with lactate formation, which under anaerobic conditions tended if anything, to increase. With increasing age the O_2-uptake successiv ly decreased between the 19th and the 25th day of the cycle. This was also true for the two oxireductases during the entire s-phase, in which the cytochrome oxidase proved the better indicator for age. This decrease with age of the three parameters of oxidative metabolism may indicate that the formation of ATP in the aging nidation medium decreases. It must be borne in mind that a given number of glucose molecules in the aerobic pathway yields about 20 times more ATP than on anaerobic glycolysis.

Neither in the p-phase nor in the s-phase did

more substrate-linked in the proliferative than in the secretory endometrium.

Judging from the direct correlation between the glucose utilisation and the formation of lactate, under aerobic conditions about 1.2 mol of lactic acid forms from 1 mol of glucose consumed, and under anaerobic conditions, about 1.6 mol these figures *remained fairly constant* throughout the cycle. As expected not only the formation of lactate but also glucose utilisation was higher under anaerobic than under aerobic conditions. In physiological environments, i.e. 5.5 mM in vitro about 20–30% of added glucose will be converted to lactic acid in the human endometrium under optimal conditions, i.e. under anaerobic conditions in the *s* phase the conversion will be 50 Since 2 mol of lactic acid can be formed from 1 mol of glucose the degree of glycolysis in the human endometrium appears fairly low However under aerobic conditions, the formation of lactate from endogenous substrates in the *s* phase was strikingly high.

The curves for the formation of lactate and LDH activity through the menstrual cycle were similar and they varied equally and significantly with the phases of the cycle. This suggests that in addition to the observed substrate linkage lactate dehydrogenase also has a governing influence on glycolysis.

As in the formation of lactate the O_2-uptake from the endogenous substrate of the endometrium increased from the *p*- to the *s*-phase However when additional amounts of glucose were added to the medium the difference between the phases of the cycle became smaller as a consequence of respiratory increase in the *p*-phase and respiratory inhibition in the *s*-phase In view of the relatively high aerobic glycolysis the respiratory inhibition in the *s*-phase was interpreted as a Crabtree effect of the endometrium (3 7).

In various glucose concentrations in the medium, the oxidation of glucose-C-6 which is a measure of the turnover through the citric acid cycle, varied inversely with the rest of the metabolic features investigated This oxidation in the *s* phase was thus lower which might have been due either to a high endogenous glucose metabolism (large glycogen pool) or to a high lipoid or protein metabolism in this phase The absolute values found for glucose-C-6-oxidation thus allowed no conclusion about the true order of the glucose breakdown through the citric acid cycle

In another series of biopsies glucose-C-6-oxidation and glucose-C-1-oxidation were compared (1-C-CO_2 minus 6-C-CO_2) (14). Since only 1-C labelled glucose can be oxidased in pentose-*phosphate shunt it was possible in this way to* assess the absolute size of the shunt in the endometrium (2) The experiments were performed with 0.5 and 5.5 mM glucose in the medium. It was found that the shunt corresponding to the highest mitotic activity was highest during late proliferation. At the same time, however the variation of the values measured was also widest. The comparison of the values found for the two glucose concentrations of the medium revealed that the shunt was substrate linked between these concentrations. Compared with the total glucose metabolism of the endometrium that of the shunt was very small These results of the maximum substrate breakdown by the pentose phosphate shunt in the late *p*-phase are incompatible with the enzyme activities of the shunt found by Hughes et al. (19) as well as by Luh & Brandau (23) These appear to be too high to limit the shunt

In order to elucidate the redox capacity of the endometrium the two oxireductases, succinate dehydrogenase and cytochrome oxidase were determined in a certain number of cases. In previous animal experiments it had been shown that these two enzymes could be influenced by steroid hormones (5 10 1) It was found that both enzyme activities—like the O uptake—increased from the *p*- to the *s*-phase

In secretory endometrium the quantitatively determined glycogen content is six times as high as in the *p*-phase (13) Determination of the breakdown of the glycogen in vitro showed that this increases successively during the cycle to reach a metabolic peak between the 19th and the 25th day with a simultaneous decrease of the relative breakdown of glycogen i.e. the relation between the amount of glycogen present and that broken down This suggests a dual function of the glycogen in the secretory endometrium It contributes to an increase and thereby stabilisation of metabolism of the endogenous substrate and also acts as an energy reserve for development of a placenta if necessary Paired deter

minations showed the variation of the glycogen *content in* the s-phase to vary with anaerobic glycolysis. The same study with endogenous respiration showed, if anything, a negative correlation which may probably be explained by the known competition between glycolysis and respiration.

With the aid of various large doses of progesterone and the administration of oestradiol to patients in the p-phase, the synthesis of glycogen in the endometrium was studied experimentally (15). *It was found that initial glycogen synthesis* does not vary with the dose of progesterone given. The synthesis increased through the p-phase in which the progesterone was given but was independent of dosage. This thus suggests the strik*ing effect of oestrogen priming resp. of the* oestrogen level. Application of 25 mg progesterone which, judging from the excretion of pregnandiol, corresponds to the physiological progesterone level in the secretory phase, did not *increase the* O_2*-uptake, but it did increase the* formation of lactate in the endometrium from the endogenous substrates as well as in the presence of glucose in the medium.

Unlike progesterone oestradiol did not increase endometrial glycogen synthesis. *As suggested by* Hughes et al. 1969 (18) and Vasquez et al. 1968 (31) the synthesis of glycogen is probably initiated by progesterone, but the rate of synthesis is controlled by oestrogens.

According to Hughes *et al. 1969 (18)*, progesterone stimulates phosphorylase activity and, judging from the present results, also the formation of lactate from glycogen. Bearing in mind the relatively constant glycogen level during the secretory phase (13), one might assume *that the* maximum secretion of progesterone during the implantation phase has the function of breaking down the glycogen faster while at the same time the oestrogens have the function of synthesising glycogen in increased amounts. In other words, this would mean a larger *passage of glycogen* during the proper secretory phase than during the presecretory phase and this would favour endogenous glycolysis at the time for implantation.

VARIOUS CATEGORIES OF ENDOMETRIUM

Routine endometrial specimens were compared with those from women with menstrual disorders and women with myomata (13). Such an investigation was considered a desirable preliminary to a planned later comparison between the endometrium from women using and not using oral contraceptives.

No difference were found between the glucose metabolism and lactate formation in the various categories of endometrium. On the other hand, differences were found in the O_2-uptake and glucose-C-6-oxidation in that the two pathological groups showed no clear difference or no difference at all between the two cyclic phases. Such a finding could be a manifestation of disturbed oxidative breakdown of glucose owing to disturbed vascularisation, possibly also as a consequence of local differences in metabolism and compensation mechanisms in an inadequately developed functional layer. Judging from the observations of Schmidt-Mathiesen 1965 (27), one would have expected an abnormal glycogen content in patients with bleeding anomalies. But this *was not the case, possibly because the bleeding* anomaly had ceased at the time of curettage. Evidence of a changed glycogen content in various gynaecological diseases may therefore be difficult to obtain because glycogen can probably be con*verted with the mucopolysaccharides.*

GLUCOSE METABOLISM AND AGE

Glucose metabolism was studied for variation with age on the basis of three groups (*≤ 35, ≥ 36–45, ≥ 46* years) and continuous age curves (15). It was found that the glucose consumption in the secretory endometrium in women up to 46 years was decreased. But this was not the case with lactate *formation, which under anaerobic conditions* tended, if anything, to increase. With increasing age the O_2-uptake successively decreased between the 19th and the 25th day of the cycle. This was also true for the two oxireductases during the entire *s-phase, in which the cytochrome oxidase* proved the better indicator for age. This decrease with age of the three parameters of oxidative metabolism may indicate that the formation of ATP in the aging oxidation medium decreases. It must be borne in mind that a *given number of* glucose molecules in the aerobic pathway *yields* about 20 times more ATP than on anaerobic glycolysis.

Neither in the p-phase nor in the s-phase did

more substrate linked in the proliferative than in the secretory endometrium.

Judging from the direct correlation between the glucose utilisation and the formation of lactate under aerobic conditions about 1 2 mol of lactic acid forms from 1 mol of glucose consumed, and under anaerobic conditions, about 1 6 mol these figures remained fairly constant throughout the cycle As expected, not only the formation of lactate but also glucose utilisation was higher under anaerobic than under aerobic conditions. In physiological environments, i.e. 5.5 mM in vitro, about 20–30% of added glucose will be converted to lactic acid in the human endometrium under optimal conditions, i.e. under anaerobic conditions in the s phase the conversion will be 50% Since 2 mol of lactic acid can be formed from 1 mol of glucose the degree of glycolysis in the human endometrium appears fairly low However under aerobic conditions the formation of lactate from endogenous substrates in the s-phase was strikingly high

The curves for the formation of lactate and LDH activity through the menstrual cycle were similar and they varied equally and significantly with the phases of the cycle This suggests that in addition to the observed substrate linkage lactate dehydrogenase also has a governing influence on glycolysis.

As in the formation of lactate the O_2-uptake from the endogenous substrate of the endometrium increased from the p- to the s-phase. However when additional amounts of glucose were added to the medium the difference between the phases of the cycle became smaller as a consequence of respiratory increase in the p-phase and respiratory inhibition in the s-phase In view of the relatively high aerobic glycolysis the respiratory inhibition in the s-phase was interpreted as a Crabtree effect of the endometrium (3 7).

In various glucose concentrations in the medium, the oxidation of glucose-C-6 which is a measure of the turnover through the citric acid cycle varied inversely with the rest of the metabolic features investigated. This oxidation in the s-phase was thus lower which might have been due either to a high endogenous glucose metabolism (large glycogen pool) or to a high lipoid or protein metabolism in this phase. The absolute values found for glucose-C-6-oxidation thus allowed no conclusion about the true order of the glucose breakdown through the citric acid cycle.

In another series of biopsies glucose-C-6-oxidation and glucose-C 1-oxidation were compared (1-C-CO_2 minus 6-C-CO) (14) Since only 1-C labelled glucose can be oxidased in pentose-phosphate shunt it was possible in this way to assess the absolute size of the shunt in the endometrium (2) The experiments were performed with 0.5 and 5.5 mM glucose in the medium. It was found that the shunt corresponding to the highest mitotic activity was highest during late proliferation. At the same time, however the variation of the values measured was also widest. The comparison of the values found for the two glucose concentrations of the medium revealed that the shunt was substrate linked between these concentrations. Compared with the total glucose metabolism of the endometrium that of the shunt was very small These results of the maximum substrate breakdown by the pentose phosphate shunt in the late p-phase are incompatible with the enzyme activities of the shunt found by Hughes et al (19) as well as by Luh & Brandau (23) These appear to be too high to limit the shunt.

In order to elucidate the redox capacity of the endometrium the two oxireductases, succinate dehydrogenase and cytochrome oxidase were determined in a certain number of cases. In previous animal experiments it had been shown that these two enzymes could be influenced by steroid hormones (5 10, 12). It was found that both enzyme activities—like the O uptake—increased from the p- to the s-phase

In secretory endometrium the quantitatively determined glycogen content is six times as high as in the p-phase (13) Determination of the breakdown of the glycogen in vitro showed that this increases successively during the cycle to reach a metabolic peak between the 19th and the 25th day with a simultaneous decrease of the relative breakdown of glycogen i e the relation between the amount of glycogen present and that broken down This suggests a dual function of the glycogen in the secretory endometrium. It contributes to an increase and thereby stabilisation of metabolism of the endogenous substrate and also acts as an energy reserve for development of a placenta if necessary Paired deter

phase and that it increases further towards the end of the cycle. After nidation in the superficial functional layer on the 13th postovulatory day the syncytiotrophoblast, according to Böving (6) is still without vascular supply for a further 7 days. Survival of this avascular phase of the syncytiotrophoblast requires high anaerobiosis, hich in the present investigation was reflected by a high Pasteur effect. It is tempting to assume that even minor deviations from optimal regulation of substrates have an unfavourable effect on nidation and may result in early abortion or sterility

GLUCOSE METABOLISM AND ORAL CONTRACEPTIVES

196 biopsy specimens were obtained from women under the influence of various oral contraceptives (combined preparations) and 36 biopsies under the influence of norethisterone acetate, 0.3 mg daily (mini-pill) and were studied regarding metabolism (13) Ovulation inhibitors cause slight proliferative and secretory stimulation until the middle of the cycle After this glandular atrophy occurs and the stroma undergoes pseudodecidual degeneration Under the influence of low doses of the gestagen norethisterone acetate there was a mild gestagenic effect in the *first half of the* cycle, but little or no change *in the histological* picture during the second part (13).

It was demonstrated that under the influence of combined preparations the aerobic and the anaerobic formation of lactate as well as the O_2 uptake, were all decreased in the presence and absence of glucose in the medium, compared w th untreated secretory endometrium. No differences were found between the glucose utilisation during the two types of cycle From these data it could be calculated that in hormone-treated endometrium, about 0 6 mol of lactic acid were formed from 1 mol of glucose, which is only half of that in untreated endometrium This suggests changed utilisation of the xogenous substrate during the use of oral contracepti es.

The formation of glycogen moderately stimulated in the beginning of a hormone treated cycle the formation increases successively during the cycle and at the end of the cycle it reaches the level of untreated s-phase.

Endogenous aerobic and anaerobic lactate formation are inversely correlated to the glycogen content. The O_2 uptake from endogenous substrate was also very low and it was therefore assumed that, as in the utilisation of exogenous substrate, also the glucose stored in glycogen was utilised less in the course of the cycle. Since the glandular atrophy and pseudodecidual degenera tion of the stroma cells at the end of the cycle is an expression of gestagen predominance over the oestrogenic influence, this assumption was also used in the interpretation of the utilisation of the substrate. Thus, compared with normal conditions, the striking discrepancy between the glycogen content and the endogenous metabolism were regarded as an expression of gestagen dominance.

Under the influence of norethisterone acetate endogenous O_2 uptake in the cycle was influenced in that the metabolism of the substrate was highest in the middle of the cycle while it was inhibited in the second half of the cycle.

In the untreated cycle carbohydrate metabolism is significantly correlated with the day of the cycle In the hormone-treated cycle no such cor relation was seen and the correlations were negative and differed significantly from those for the untreated cycle. Also under the influence of nor ethisterone acetate in two series substrate correla tions were no longer demonstrable. With the ovulation inhibitors the cessation of the correlation was due partly to differences in the arious commercial preparations with which the metabolism was studied. The negati e correlation reflects partly an initial stimulation of the metabolism which is later inhibited Such a course of carbohydrate metabolism was also compatible with the histological pictures of the endometrium during the use of oral contraceptives.

The strong inhibition of carbohydrate metabolism by oral contraception (and to a certain extent also after administration of norethisterone acetate) may in the light of the observations made in a normal cycle be regarded as a contraceptiv component in the endometrium. For it may be assumed that the conditions for nidation are impaired either by changed energy supply or by a change in the pH of the endometrium

REFERENCES

1 Adler, K. Der Gewebsstoffwechsel der Uterusschleimhaut in den verschiedenen Stadien ihrer biologischen Funktion. Arch Gynäk 141 306, 1930.

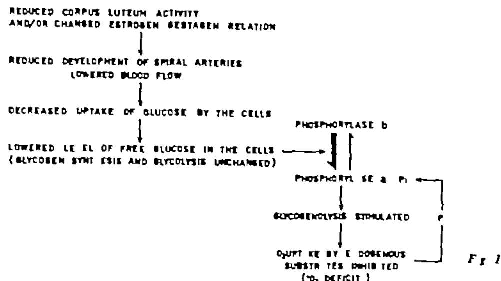


Fig. 1

the total amount of glycogen vary with age. As for the change in other parameters with age, in contrast with what has been supposed by some authors such as Randall & Power (25) Payne & Latour (24) and Hughes et al. (19) the total endometrial glycogen did not appear to be a measure of the functional condition of the endometrium.

On the basis of the present observations and other known aging changes and demonstrated biochemical relationships, the substrate in the secretory endometrium of an aging woman appears to be regulated in such a way that glucose is broken down to a greater extent via glycogen than in younger age and then as shown in Fig. 1

By this concept the occurrence of free glucose in the cell would be decreased. However this condition is well known to stimulate the conversion of the inactive phosphorylase *b* to the active *a*-form. The glycogen thereby released is again broken down via endogenous glycogenolysis, which results in inhibition of respiration from endogenous substrate.

It is very questionable whether substrate elsewhere can contribute to respiration because the redox capacity is probably limited. Therefore less inorganic phosphate will be consumed in the respiratory chain whereby this will be at the disposal of the phosphorylase *a* which is in need of phosphate. The result will be a vicious circle However this teaches nothing about the primary cause of the change in the regulation of the substrate in the endometrium of the aging woman.

Assuming that the energy in the nidation medium of the young woman is optimal and not excessive for the nidating blastocyst the limitation of ATP-formation in the nidation medium of the aging woman might partly explain why her fertility is decreased.

REGULATION OF SUBSTRATE IN THE IMPLANTATION PHASE

The difference in the regulation of the substrate of energy metabolism prevailing between the proliferative and secretory endometrium shown by Hackl 1970 (13), suggests that the endometrium prepares itself in a special way for the blastocyst. On the one hand the fact that glycolysis was not in certainty substrate-linked suggests a higher resistance to fluctuations of the glucose level this was also suggested by the high endogenous glycolysis. On the other hand, the Crabtree-effect observed suggests a special regulation of the ATP production Thus, with little glucose available respiration can increase to form a definite amount of ATP while when more glucose is available it can have an inhibitory effect to keep the amount of ATP constant Such a regulation mechanism also makes it probable that the energy formation of the nidation medium for the blastocyst always is optimal but not excessive This would lend further support to the impression that loss of energy with advancing age contributes to the reduction of fertility of aging woman.

In the determination of the absolute Pasteur effect (anaerobic lactate minus aerobic lactate/unit) it was found that this is higher in the se-

phase and that it increases further towards the end of the cycle. After nidation in the superficial functional layer on the 13th postovulatory day the syncytiotrophoblast, according to Böving (6), is still without vascular supply for a further 7 days. Survival of this avascular phase of the syncytiotrophoblast requires high anaerobiosis, which in the present investigation was reflected by a high Pasteur effect. It is tempting to assume that even minor deviations from optimal regulation of substrates have an unfavourable effect on nidation and may result in early abortion or sterility.

GLUCOSE METABOLISM AND ORAL CONTRACEPTIVES

196 biopsy specimens were obtained from women under the influence of various oral contraceptives (combined preparations) and 36 biopsies under the influence of norethisterone acetate, 0.3 mg daily (mini-pill), and were studied regarding metabolism (13). Ovulation inhibitors cause slight proliferation and secretory stimulation until the middle of the cycle. After this glandular atrophy occurs and the stroma undergoes pseudodecidual degeneration. Under the influence of low doses of the gestagen norethisterone acetate there was a mild gestagenic effect in the first half of the cycle but little or no change in the histological picture during the second part (13).

It was demonstrated that under the influence of combined preparation the aerobic and the anaerobic formation of lactate as well as the O_2 uptake were all decreased in the presence and absence of glucose in the medium, compared with untreated secretory endometrium. No differences were found between the glucose utilisation during the two types of cycle. From these data it could be calculated that in hormone-treated endometrium, about 0.6 mol of lactic acid were formed from 1 mol of glucose, which is only half of that in untreated endometrium. This suggests a changed utilisation of the exogenous substrate during the use of oral contraceptives.

The formation of glycogen moderately stimulated in the beginning of hormone-treated cycle the formation increases successively during the cycle and at the end of the cycle it reaches the level of untreated s-phase.

Endogenous aerobic and anaerobic lactate formation are inversely correlated to the glycogen content. The O_2 uptake from endogenous substrate was also very low and it was therefore assumed that, as in the utilisation of exogenous substrate also the glucose stored in glycogen was utilised less in the course of the cycle. Since the glandular atrophy and pseudodecidual degeneration of the stroma cells at the end of the cycle is an expression of gestagen predominance over the oestrogenic influence, this assumption was also used in the interpretation of the utilisation of the substrate. Thus, compared with normal conditions, the striking discrepancy between the glycogen content and the endogenous metabolism were regarded as an expression of gestagen dominance.

Under the influence of norethisterone acetate endogenous O_2 uptake in the cycle was influenced in that the metabolism of the substrate was highest in the middle of the cycle while it was inhibited in the second half of the cycle.

In the untreated cycle carbohydrate metabolism is significantly correlated with the day of the cycle. In the hormone-treated cycle no such correlation was seen and the correlations were negative and differed significantly from those for the untreated cycle. Also under the influence of norethisterone acetate in two series substrate correlations were no longer demonstrable. With the ovulation inhibitors the cessation of the correlation was due partly to differences in the various commercial preparations with which the metabolism was studied. The negative correlation reflects partly an initial stimulation of the metabolism which is later inhibited. Such a course of carbohydrate metabolism was also compatible with the histological pictures of the endometrium during the use of oral contraceptives.

The strong inhibition of carbohydrate metabolism by oral contraception (and to a certain extent also after administration of norethisterone acetate) may in the light of the observations made in normal cycle be regarded as a contraceptive component in the endometrium. For it may be assumed that the conditions for nidation are impaired either by changed energy supply or by changes in the pH of the endometrium.

REFERENCES

1. Adler K. Der Gewebsstoffwechsel der Uterusschleimhaut in den verschiedenen Stadien ihrer biologischen Funktion. Arch Gynäk *141*: 306, 1930.

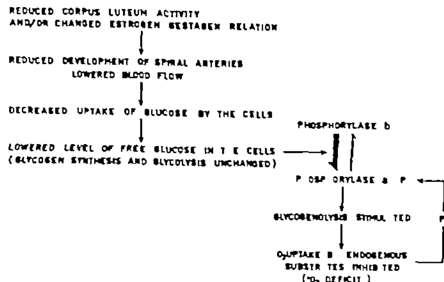


Fig 1

the total amount of glycogen vary with age. As for the change in other parameters with age in contrast with what has been supposed by some authors such as Randall & Power (25) Payne & Latour (24) and Hughes et al. (19) the total endometrial glycogen did not appear to be a measure of the functional condition of the endometrium.

On the basis of the present observations and other known aging changes and demonstrated biochemical relationships, the substrate in the secretory endometrium of an aging woman appears to be regulated in such a way that glucose is broken down to a greater extent via glycogen *than in younger age and then as shown in Fig. 1*

By this concept the occurrence of free glucose in the cell would be decreased. However this condition is well known to stimulate the conversion of the inactive phosphorylase *b* to the active *a*-form. The glycogen thereby released is again broken down via endogenous glycogenolysis, which results in inhibition of respiration from endogenous substrate

It is very questionable whether substrate elsewhere can contribute to respiration because the redox capacity is probably limited. Therefore less inorganic phosphate will be consumed in the respiratory chain whereby this will be at the disposal of the phosphorylase *a* which is in need of phosphate. The result will be a vicious circle. However this teaches nothing about *the primary* cause of the change in the regulation of the substrate in the endometrium of the aging woman.

Assuming that the energy *in* the nidation medium of the young woman is optimal and not excessive for the nidating blastocyst, the limitation of *ATP formation in the nidation medium* of the aging woman might partly explain why her fertility is decreased.

REGULATION OF SUBSTRATE IN THE IMPLANTATION PHASE

The difference in the regulation of the substrate of energy metabolism prevailing between the proliferative and secretory endometrium shown by Hackl 1970 (13) suggests that the endometrium prepares itself in a special way for the blastocyst. *On the one hand the fact that glycolysis was* not in certainty substrate-linked suggests a higher resistance to fluctuations of the glucose level this was also suggested by the high endogenous glycolysis. On the other hand, the Crabtree-effect observed suggests a special regulation of the ATP production Thus, with little glucose available respiration can increase to form a definite amount of ATP while when more glucose is available it can have an inhibitory effect to keep the amount of ATP constant Such a regulation mechanism also makes it probable that the energy formation of the nidation medium for the blastocyst *always is optimal but not excessi e This* would lend further support to the impression that loss of energy with advancing age contributes to *the reduction of fertility of aging woman.*

In the determination of the absolute Pasteur effect (anaerobic lactate minus aerobic lactate/unit) it was found that this is higher in the r-

phase and that it increases further towards the end of the cycle. After nidation in the superficial functional layer on the 13th postovulatory day the syncytiotrophoblast, according to Böving (6) is still without vascular supply for a further 7 days. Survival of this avascular phase of the syncytiotrophoblast requires high anaerobism, which in the present investigation was reflected by a high Pasteur effect. It is tempting to assume that even minor deviations from optimal regulation of substrates has an unfavourable effect on nidation and may result in early abortion or sterility.

GLUCOSE METABOLISM AND ORAL CONTRACEPTIVES

196 biopsy specimens were obtained from women under the influence of various oral contraceptives (combined preparations) and 36 biopsies under the influence of norethisterone acetate, 0.3 mg daily (mini-pill), and were studied regarding metabolism (13). Ovulation inhibitors cause slight proliferative and secretory stimulation until the middle of the cycle. After this glandular atrophy occurs and the stroma undergoes pseudodecidual degeneration. Under the influence of low doses of the gestagen norethisterone acetate there was a mild gestagenic effect in the first half of the cycle but little or no change in the histological picture during the second part (13).

It was demonstrated that under the influence of combined preparations the aerobic and the anaerobic formation of lactate as well as the O_2 uptake were all decreased in the presence and absence of glucose in the medium, compared with untreated secretory endometrium. No differences were found between the glucose utilisation during the two types of cycle. From these data it could be calculated that in hormone-treated endometrium about 0.6 mol of lactic acid were formed from 1 mol of glucose, which is only half of that in untreated endometrium. This suggests a changed utilisation of the exogenous substrate during the use of oral contraceptives.

The formation of glycogen moderately stimulated in the beginning of hormone-treated cycle; the formation increases successively during the cycle and at the end of the cycle it reaches the level of untreated s-phase.

Endogenous aerobic and anaerobic lactate formation are inversely correlated to the glycogen content. The O_2 uptake from endogenous substrate was also very low and it was therefore assumed that, as in the utilisation of exogenous substrate, also the glucose stored in glycogen was utilised less in the course of the cycle. Since the glandular atrophy and pseudodecidual degeneration of the stroma cells at the end of the cycle is an expression of gestagen predominance over the oestrogenic influence, this assumption was also used in the interpretation of the utilisation of the substrate. Thus, compared with normal conditions, the striking discrepancy between the glycogen content and the endogenous metabolism were regarded as an expression of gestagen dominance.

Under the influence of norethisterone acetate endogenous O_2 uptake in the cycle was influenced in that the metabolism of the substrate was highest in the middle of the cycle while it was inhibited in the second half of the cycle.

In the untreated cycle carbohydrate metabolism is significantly correlated with the day of the cycle. In the hormone-treated cycle no such correlation was seen and the correlations were negative and differed significantly from those for the untreated cycle. Also under the influence of norethisterone acetate in two series substrate correlations were no longer demonstrable. With the ovulation inhibitors the cessation of the correlation was due partly to differences in the various commercial preparations with which the metabolism was studied. The negative correlation reflects partly an initial stimulation of the metabolism which is later inhibited. Such a course of carbohydrate metabolism was also compatible with the histological pictures of the endometrium during the use of oral contraceptives.

The strong inhibition of carbohydrate metabolism by oral contraception (and to a certain extent also after administration of norethisterone acetate) may, in the light of the observations made in a normal cycle, be regarded as a contraceptive component in the endometrium. For it may be assumed that the conditions for nidation are impaired either by changed energy supply or by a change in the pH of the endometrium.

REFERENCES

1. Adler, L.: Der Glykogengehalt der Uterusschleimhaut in den verschiedenen Stadien ihrer biologischen Funktion. Arch. Gynäk. *141*, 306, 1930.

2. Agranoff B. W Brady R. O & Colodzin, M Differential conversion of special labelled glucose to $^{14}CO_2$. J Biol Chem *11* 773 1954
3 Aisenberg, A. C. The Glycolysis and Respiration of Tumors. Academic Press, New York, 1961
4 Barton, R. R. A specific method for quantitative determination of glucose Analyt Biochem *14* 258, 1966.
5 Biddulph, C. M San Martin, M Becker S. C. & McShan, W H Succinic dehydrogenase and adenosintriphosphatase activities of corpora lutea, ovarian interstitial tissue and endometrium from pseudopregnant rabbits treated with gonadotrophic hormones. J Endocrin *18* 125 1959
6. Böving, B G The biology of trophoblast. Ann NY Acad Sci *80* 21 1959
7 Crabtree H G Observations on the carbohydrate metabolism of tumors. Biochem J 3 536, 1929
8. Dományi S., Székely J & Tóth, K. Metabolism of normal and pathological human endometrium. Acta Phys Acad Sci Hung 3 1 3 1967
9 Dreyfuss, M L The metabolism of human endometrium with special reference to adenomyosis and hyperplasia of endometrium. Am J Cancer *38* 551 1940.
10. Eckstein, B. & Villee C. A. Effect f estradiol on enzymes of carbohydrate metabolism in rat uterus. Endocrinology *78* 409 1966
11 Eschbach, W & Negelein, E. Über den Stoff echsel des Endometriums. Arch Gynak *187* 337 1956.
12. Fawzy S., Mansour M D & Barady A F Enzyme histochemical study on postmenopausal endometrium in ymptom free patients. Am J Obstet Gynec *97* 109 1967
13 Hackl, H Studien des in vitro-Glucosestoffwechsels humaner Endometrien mit Rücksicht auf den normalen und artefiziellen Zyklus. Academic dissertation, Lund, 1970.
14 — Zum Pentose Phosphat-Shunt im humanen Endometrium. Arch Gynäk *11* 6?7 1971
15 — Zur hormonellen Steuerung der Glykogensynthese im humanen Endometrium. Arch Gynäk *11* 617 1971
16. Hagerman, D D & Villee C A. Effects of the menstrual cycle on the metabolism of human endometrium. Endocrinology *53* 667 1953
17 Hess, B Über eine kinetisch-enzymatische Bestimmung der *L(+)-M* lchsäure im menschlichen Serum und anderen biologischen Flüssigkeiten. Biochemie 3 8 110, 1956.
18. Hughes, E. C., Demers, L. M Csermely T & Jones, D B Organ culture of human endometrium Effect of ovarian steroids. Amer J Obstet Gynec *105* 707 1969
19 Hughes, E. C Jacobs, R. D Rubulis, A. & Husney, R. M Carbohydrate pathways of the endometrium. Effects on ovular growth. Amer J Obstet Gynec *85* 594 1963
20. Hughes, E. C., Jacobs, R. D & Rubulis, A. Effect of treatment for sterility and abortion upon the carbohydrate pathways of the endometrium. Amer J Obstet Gynec *89* 59 1964
21 Hughes, E. C., van Ness, A. W & Lloyd, C. W The nutritional value of the endometrium for implantation and in habitual abortion. Amer J Obstet Gynec *59* 129., 1950.
22. Kunz H W Über die Stoffwechselwirkung der weiblichen Geschlechtshormone Dissertation, Tubingen, 1951
23 *Luh, W & Brandau, H Enzymologische Studien am* normalen menschlichen Endometrium. Z Geburtsh Gynak *168* 14 1967
24 Payne H W & Latour J P A. Quantitative estimations of endometrial glycogen, using the Anthrone method. J Clin Endocr *15* 1106, 1955
25 Randall, M & Power M H. Amounts of glycogen in the endometrium. Proc Mayo Clin *17* 158 1942.
26. Rerup, C. & Lundquist, I Non-specific reaction of current glucose oxidase preparations with glycogen and its application for glycogen determinations in tissue Acta Pharmacol T xicol 25 41 1967
27 Schmidt Mathiesen, H. Die dysfunktionelle uterine Blutung. Histochemie und Mechanismus. Gynaecologia *160* 197 1965
28 Schwartz, M K, Kessler G & Bodansky O. Automated assay f activities of enzymes involving the diphosphopyridine nucleotide reduced diphosphopyridine nucleotide reaction. J Biol Chem 36 1?07 1961
29 St rmer V M & Stein, R. J Cytodynamic properties f the human endometrium. V Metabolism and enzymat c act v ty of the human endometrium during the menstrual cycle Am J Obstet Gynec *63* 359 195.
30. Umbreit, W W Burris, R. H & Stauffer J F Manometric Techniques. Burgess Publishing Co Minneapolis, 1959
31 Vázquez, E Rovalo, J & García, G. Comparison of progestional ffect of progesterone and chlormadinone progressiv doses on human endometrium Int J Fertil *13* 103 1968.

Submitted for publicat on J ne ?6 197

Heinrich Hackl
K innokliniken
451 00 Uddevalla
S eden

Acta Obstet Gynec Scand 52, 141–145 1973

FIBRIN DEGRADATION PRODUCTS DURING POSTOPERATIVE RADIOTHERAPY OF OVARIAN CARCINOMA

Lars Svanberg, Birger Åstedt, Inge Gynning and Inga Marie Nilsson

From the Coagulation Laboratory (Head: Professor Inga Marie Nilsson), the Department of Obstetrics and Gynecology (Head: Professor Stig Kullander) and the Department of Radiotherapy (Head: Inge Gynning, M.D.), Malmö Allmänna Sjukhus, Malmö, Sweden

Abstract. Determinations were made of fibrin degradation products (FDP) in serum from 19 patients who were receiving radiotherapy after the surgical treatment of malignant ovarian tumours. FDP decreased or disappeared in association with operation and radiotherapy but increased or re-appeared with recurrences. Possible explanations of this phenomenon are discussed.

Malignant tumours have coagulative properties (13) and require a fibrin network as a matrix for growth and vascularization (9). Such neoplasms also possess fibrinolytic activity (4, 5, 6, 15, 17). The activity of such coagulative and fibrinolytic processes results in the occurrence of fibrinogen/fibrin degradation products (FDP) in the blood. Åstedt et al. (19) thus found FDP in the serum from 72% of patients with malignant ovarian tumours. In a case of malignant ovarian androblastoma we also observed disappearance of FDP in association with radiotherapy (20).

This paper concerns the behaviour of FDP during the postoperative course with radiotherapy of malignant ovarian tumours and the level of such products during follow-up.

MATERIAL AND METHODS

The clinical material consisted of 19 patients receiving radiotherapy after surgical operation because of malignant ovarian tumours. *Determinations* were made of the FDP before operation and at regular intervals during radiotherapy after operation, and during follow-up. Histological examination of operative specimens had verified the diagnosis in all cases. In 7 cases also ascitic fluid was examined for FDP and in 6 cases cyst fluid from the tumour.

The material was divided into three groups.

Group I consisted of 6 patients in whom all malignant tissue seen at operation was removed but the patient also had small amounts of ascites. The patients received postoperative radiotherapy as a rule external roentgen radiation, of the pelvis as well as of the epigastrium. The dose, 2 000 to 3 200 R skin dose, was delivered to the pelvis via 2 or 3 anterior abdominal fields and 2 posterior pelvic fields. As a rule a skin dose of 2 000–3 200 R was also delivered to two epigastric fields. When the largest dose was used it was given in two series at an intermediate interval of 3–4 weeks. None of the patients had clinical recurrence during a follow-up period of 6–12 months.

Group II consisted of 4 cases with macroscopic extraovarian secondaries and ascites. The operation in these cases was not radical. These 4 patients were given more intensive radiotherapy with high voltage irradiation of the pelvis with a dose of about 6 000 rad at a rate of about 200 rad per day of treatment. In addition, a total skin dose of 2 000–3 200 R was delivered via two fields in the epigastrium. All 4 patients had recurrences.

Group III consisted of 9 cases judged as inoperable. Radiotherapy in these cases was merely palliative and was, as a rule, discontinued after the patient had received only relatively small doses owing to her poor general condition.

Collection of blood. Blood was collected in tubes containing an inhibitor of fibrinolysis, EACA, to prevent in vitro fibrinolysis, as well as thrombin to prevent incomplete coagulation with residual fibrinogen (30 NIH units thrombin and 25 mg EACA to 3 ml blood). Serum from these samples was prepared in the way described by Nilehn (11). *Ascites* and *cyst fluid* was also collected with the addition of EACA and thrombin, but not centrifuged.

Determination of FDP. FDP were determined by the immunochemical method of Nilehn (11). In this method an antiserum against the D fraction of the FDP is applied to agarose gel. With high voltage electrophoresis serum migrates into the gel. If FDP (X, Y or D products) are present, they will produce precipitation peaks. The height of such peaks is measured and related to a standard of substances of high molecular weight. The method measures FDP down to a concentration of 5 μg/ml. In the

Table I. *Preoperative determination of FDP in serum in patients with malignant ovarian tumours*

	Present	Absent
Group I	3	3
Group II	4	0
Group III	9	0
Total	16	3 – 19

presence of aminocaproic acid and thrombin this method will not show any FDP in the serum of healthy control women even in repeated assays.

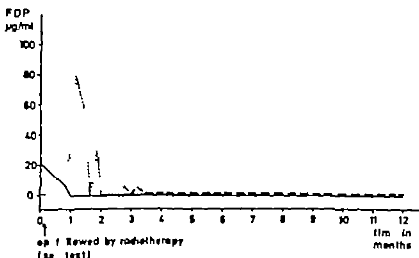
RESULTS

FDP were demonstrated preoperatively in 16 (84 %) of the 19 cases (Table I)

In group I (6 cases) where surgical treatment and radiotherapy appeared satisfactory and in which no signs of a recurrence have been observed during follow-up, the FDP first increased and afterwards disappeared within one to three months. FDP did not re-appear (Figs. 1 *a* and *b*).

In group II (4 cases) in which the operation was not radical but in which a complete course of radiotherapy had been given and in which recurrences appeared during follow-up the FDP first showed a transient increase and then disappeared *until the development of a recurrence* (Fig 2 *a*). In two patients FDP were not demonstrable until *after* palpation had revealed a recurrence, while in two cases the appearence of FDP had *antedated* the clinical symtoms of a recurrence. One of the cases is described in further detail (10).

A girl born in 1953 and subjected to rightsided salpingo-oophorectomy in June 1969 because of an ovarian androblastoma of uncertain degree of malignancy. Six months later she was re-admitted because of a large palpable recurrence. Analysis revealed FDP in concentration of 100 µg/ml. After radiotherapy alone the abdominal mass was no longer palpable and FDP were no longer demonstrable. In June 1970 a new recurrence was diag-



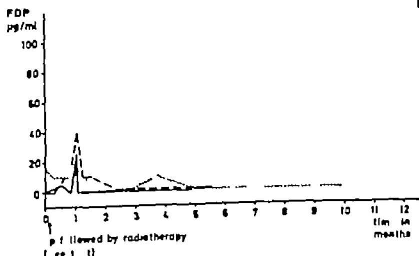


Fig 1

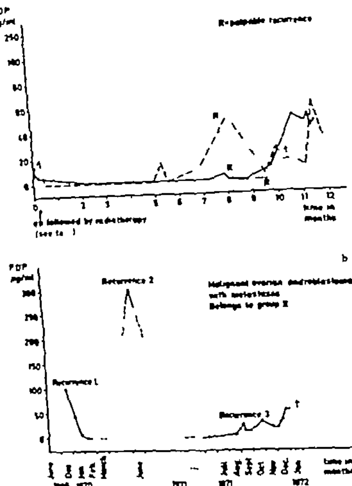


Fig. 2

moved and the FDP concentration was found to be 300 µg/ml. After irradiation and surgical excision of all visible malignant tissue the FDP disappeared. No FDP were afterwards demonstrable until August 1971, when a third recurrence was detected. Despite reoperation, radiation and treatment with cytostatic drugs the tumour grew rapidly and the patient died in January 1972. The FDP concentration was then 90 µg/ml (Fig. 2 *b*).

In group III (9 cases) where removal of the tumour had proved impossible and where radiotherapy was entirely palliative the high level of FDP persisted until the patients died (Figs. 3 *a* and *b*).

FDP were also determined in cyst fluid obtained from 6 malignant tumours at the operation. In these cases the levels ranged from 1 500 to 7 000 µg/ml.

In 7 cases all belonging to group III, FDP were also determined in ascitic fluid in which it ranged from 1 000 and 3 500 µg/ml. In one patient FDP were determined in ascitic fluid before and after a complete course of radiotherapy. At the first explorative laparotomy the concentration of FDP in ascitic fluid was 3 500 µg/ml. At re-operation after radiotherapy the concentration of FDP in ascitic fluid was only 25 µg/ml. In patients, who received a smaller palliative dose of radiotherapy FDP in ascitic fluid did not notably decrease.

DISCUSSION

It is not known exactly why FDP occur in the blood in patients with malignant tumours. O'Meara (13) showed that malignant tissue has coagulative properties. He also demonstrated a clot-forming enzyme in such tumours. Laki & Yancey

Table I *Preoperative determination of FDP in serum in patients with malignant ovarian tumours*

	Present	Absent
Group I	3	3
Group II	4	0
Group III	9	0
Total	16	3 = 19

presence of aminocaproic acid and thrombin this method will not show any FDP in the serum of healthy control women even in repeated assays.

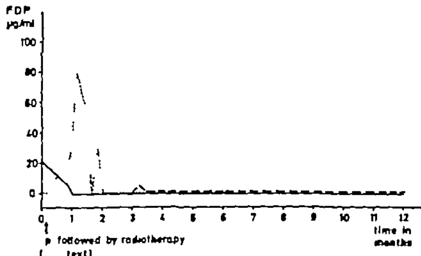
RESULTS

FDP were demonstrated preoperatively in 16 (84 %) of the 19 cases (Table I)

In group I (6 cases) where surgical treatment and radiotherapy appeared satisfactory and in which no signs of a recurrence have been observed during follow-up the FDP first increased and afterwards disappeared within one to three months. FDP did not re appear (Figs. 1 *a* and *b*).

In group II (4 cases) in which the operation was not radical but in which a complete course of radiotherapy had been given and in which recurrences appeared during follow up the FDP first showed a transient increase and then disappeared until the development of a recurrence (Fig 2 *a*). *In two patients FDP were not demonstrable* until *after* palpation had revealed a recurrence while in two cases the appearence of FDP had *antedated* the clinical symtoms of a recurrence. One of the cases is described in further detail (20).

A girl born in 1953 and subjected to rightsided salpingo-oophorectomy in June 1969 because of an ovarian androblastoma of uncertain degree of malignancy Six months later she was re-admitted because of a large palpable recurrence Analysis revealed FDP in concentration of 100 µg/ml. After radiotherapy alone, the abdominal mass was no longer palpable and FDP were no longer demonstrable In June 1970 a new recurrence was diag-



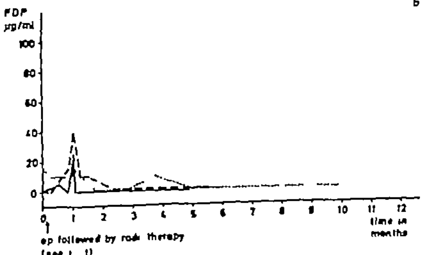


Fig 1

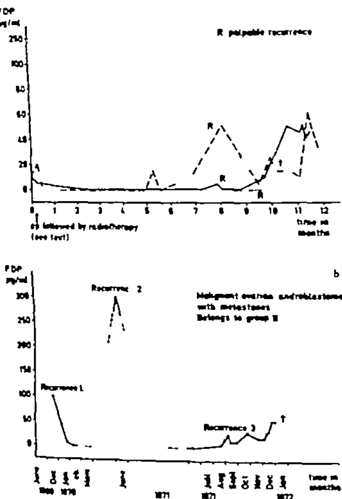


Fig. 2

tored and the FDP concentration was found to be 300 µg/ml. After irradiation and surgical excision of all visible malignant tissue the FDP disappeared. No FDP were afterwards demonstrable until August 1971 when third recurrence was detected. Despite reoperation, radiation and treatment with cytostatic drugs the tumour grew rapidly and the patient died in January 1972. The FDP concentration was then 50 µg/ml (Fig. 2 *b*).

In group III (9 cases) where removal of the tumour had proved impossible and where radiotherapy was entirely palliative the high level of FDP persisted until the patients died (Figs. 3 *a* and *b*).

FDP were also determined in cyst fluid obtained from 6 malignant tumours at the operation. In these cases the levels ranged from 1 500 to 7 000 µg/ml.

In 7 cases all belonging to group III FDP were also determined in ascitic fluid in which it ranged from 1 000 and 3 500 µg/ml. In one patient FDP were determined in ascitic fluid before and after a complete course of radiotherapy. At the first explorative laparotomy the concentration of FDP in ascitic fluid was 3 500 µg/ml. At re-operation after radiotherapy the concentration of FDP in ascitic fluid was only 25 µg/ml. In patients, who received a smaller palliative dose of radiotherapy FDP in ascitic fluid did not notably decrease.

DISCUSSION

It is not known exactly why FDP occur in the blood in patients with malignant tumours. O'Meara (13) showed that malignant tissue has coagulative properties. He also demonstrated a clot-forming enzyme in such tumours. Laki & Yancey

Table I *Preoperative determination of FDP in serum in patients with malignant ovarian tumours*

	Present	Absent
Group I	3	3
Group II	4	0
Group III	9	0
Total	16	3 = 19

presence of aminocaproic acid and thrombin this method will not show any FDP in the serum of healthy control women even in repeated assays.

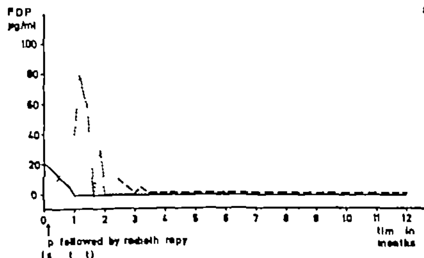
RESULTS

FDP were demonstrated preoperatively in 16 (84%) of the 19 cases (Table I)

In group I (6 cases) where surgical treatment and radiotherapy appeared satisfactory and in which no signs of a recurrence have been observed during follow-up, the FDP first increased and afterwards disappeared within one to three months. FDP did not re-appear (Figs. 1 *a* and *b*)

In group II (4 cases) in which the operation was not radical but in which a complete course of radiotherapy had been given and in which recurrences appeared during follow-up the FDP first showed a transient increase and then disappeared until the development of a recurrence (Fig. 2 *a*) In two patients FDP were not demonstrable until *after* palpation had revealed a recurrence while in two cases the appearence of FDP had *antedated* the clinical symtoms of a recurrence. One of the cases is described in further detail (10).

A girl born in 1953 and subjected to right-sided salpingo-oophorectomy in June 1969 because of an ovarian androblastoma of uncertain degree of malignancy Six months later she was re-admitted because of a large palpable recurrence Analysis revealed FDP in concentration of 100 µg/ml. After radiotherapy alone, the abdominal mass was no longer palpable and *FDP* were *no longer* demonstrable In June 1970 a new recurrence was diag-



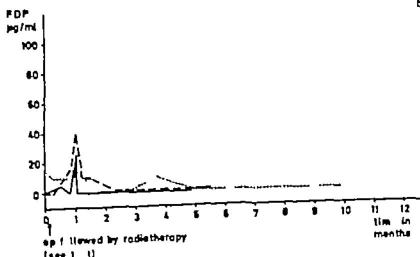
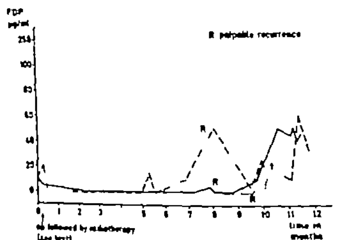


Fig 1



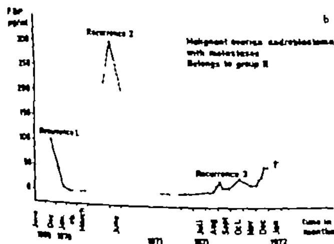


Fig. 2

...ved and the FDP concentration was found to be 300 μg/ml. After irradiation and surgical excision of all visible malignant tissue the FDP disappeared. No FDP were afterwards demonstrable until August 1971 when third recurrence was detected. Despite reoperation, radiation and treatment with cytotoxic drugs the tumour grew rapidly and the patient died in January 1972. The FDP concentration was then 50 μg/ml (Fig. 2 *b*).

In group III (9 cases) where removal of the tumour had proved impossible and where radiotherapy was entirely palliative the high level of FDP persisted until the patients died (Figs. 3 *a* and *b*).

FDP were also determined in cyst fluid obtained from 6 malignant tumours at the operation. In these cases the levels ranged from 1 900 to 7 000 μg/ml.

In 7 cases all belonging to group III FDP were also determined in ascitic fluid in which it ranged from 1 000 and 3 500 μg/ml. In one patient FDP were determined in ascitic fluid before and after a complete course of radiotherapy. At the first explorative laparotomy the concentration of FDP in ascitic fluid was 3 500 μg/ml. At re-operation after radiotherapy the concentration of FDP in ascitic fluid was only 25 μg/ml. In patients, who received a smaller palliative dose of radiotherapy FDP in ascitic fluid did not notably decrease.

DISCUSSION

It is not known exactly why FDP occur in the blood in patients with malignant tumours. O'Meara (13) showed that malignant tissue has coagulative properties. He also demonstrated a clot forming enzyme in such tumours. Laki & Yancey

Table I *Preoperative determination of FDP in serum in patients with malignant ovarian tumours*

	Present	Absent
Group I	3	3
Group II	4	0
Group III	9	0
Total	16	3 – 19

presence of aminocaproic acid and thrombin this method will not show any FDP in the serum of healthy control women even in repeated assays.

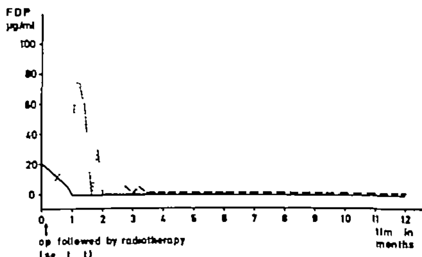
RESULTS

FDP were demonstrated preoperatively in 16 (84 %) of the 19 cases (Table I)

In group I (6 cases) where surgical treatment and radiotherapy appeared satisfactory and in which no signs of a recurrence have been observed during follow-up, the FDP first increased and afterwards disappeared within one to three months. FDP did not re-appear (Figs. 1 *a* and *b*).

In group II (4 cases) in which the operation was not radical but in which a complete course of radiotherapy had been given and in which recurrences appeared during follow-up the FDP first showed a transient increase and then disappeared until the development of a recurrence (Fig. 2 *a*). In two patients FDP were not demonstrable until *after* palpation had revealed a recurrence, while in two cases the appearence of FDP had *antedated* the clinical symtoms of a recurrence. One of the cases is described in further detail (20).

A girl born in 1953 and subjected to rightsided salpingo-oophorectomy in June 1969 because of an ovarian androblastoma of uncertain degree of malignancy. Six months later she was re-admitted because of a large palpable recurrence. Analysis revealed FDP in concentration of 100 µg/ml. After radiotherapy alone the abdominal mass was no longer palpable and FDP were no longer demonstrable. In June 1970 a new recurrence was diag-



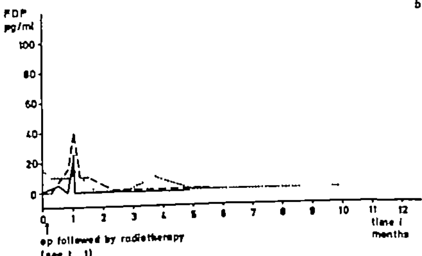


Fig. 1

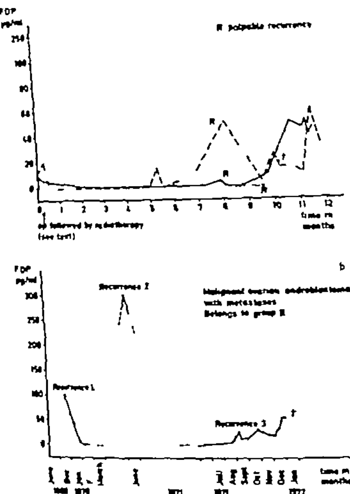


Fig. 2

...ased and the FDP concentration was found to be 300 μg/ml. After irradiation and surgical removal of all visible malignant tissue the FDP disappeared. No FDP were afterwards demonstrable until August 1971, when a third recurrence was detected. Despite reoperation, radiation and treatment with cytotoxic drugs the tumour grew rapidly and the patient died in January 1972. The FDP concentration was then 50 μg/ml (Fig. 2 *b*).

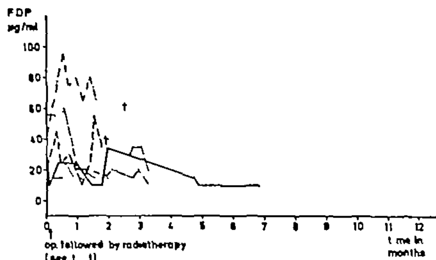
In group III (9 cases) where removal of the tumour had proved impossible and where radiotherapy was entirely palliative the high level of FDP persisted until the patients died (Figs. 3 *a* and *b*).

FDP were also determined in cyst fluid obtained from 6 malignant tumours at the operation. In these cases the levels ranged from 1 500 to 7 000 μg/ml.

In 7 cases all belonging to group III FDP were also determined in ascitic fluid in which it ranged from 1 000 and 3 500 μg/ml. In one patient FDP were determined in ascitic fluid before and after a complete course of radiotherapy. At the first explorative laparotomy the concentration of FDP in ascitic fluid was 3 500 μg/ml. At re-operation after radiotherapy the concentration of FDP in ascitic fluid was only 25 μg/ml. In patients, who received a smaller palliative dose of radiotherapy FDP in ascitic fluid did not notably decrease.

DISCUSSION

It is not known exactly why FDP occur in the blood in patients with malignant tumours. O'Meara (13) showed that malignant tissue has coagulative properties. He also demonstrated a clot forming enzyme in such tumours. Lakl & Yancey



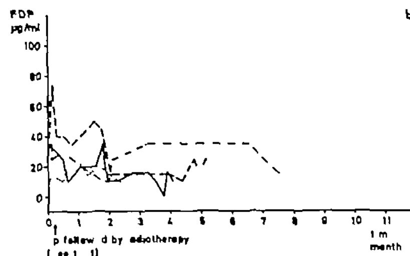


Fig 3

(9) analysed this clot-forming enzyme in tumour extracts and found that it was thrombin. They thought that a fibrin network is necessary for vascularisation and growth of tumours. It has long been known that malignant tissue can dissolve fibrin (2 16) Cliffton & Grossi (5) also showed that mesenchymal tumours exhibit a higher activity on fibrin plates than epithelial tissue Fibrinolytic activity in malignant tissue has also been demonstrated histochemically (4 6, 15).

FDP are demonstrable in blood from patients with generalised fibrinolysis, intravascular coagulation with secondary fibrinolysis or local fibrin deposits with secondary fibrinolysis. Malignant tumours can cause such conditions because of their coagulative and fibrinolytic properties and give rise to FDP In a previous series (19) consisting of 163 patients with palpable ovarian masses we found FDP in the serum from 72 % of those tumours which surgery revealed to be malignant

In the present material FDP were demonstra[ble] preoperatively in 84 % An interesting observat[ion] made in the present investigation was that the concentration of FDP in the serum was influenced by radiotherapy

In all the 19 patients the FDP level first rose within 3–4 weeks after the beginning of radiotherapy This increase can probably be ascribed to destruction of tissue and vessels with the occurrence of thromboplastic substances and/or fibrinolytic enzymes in the bloodstream (10) There is no reason to ascribe this increase to the operation since Hedner & Nilsson (8) have shown that even after major operations only small amounts of FDP are demonstrable in the blood and then only during the first week after the operation

The FDP then decreased or disappeared. Various explanations may be offered for this late decrease or disappearance of FDP For example

radiotherapy causes several degenerative changes in the irradiated tissue, and interferes especially with the enzyme processes in the cells. It has been shown histochemically (14 18) that the fibrinolytic activity of the blood vessels in a tumour (the fibrinolytic activators are localised mainly to the vasa vasorum of the adventitia (12)) is related to its vascularity (2, 3). Irradiation of animals has been shown to produce changes in all layers of the vessel walls, but most in the adventitia because of its relatively high collagen content (7). Large doses result in hyalinisation of the collagen fibers. Even relatively small doses cause swelling of the endothelial cells and reduction of their ability to form new capillaries. The adventitia of the vessel walls has thus found to be missing in all animals killed 4–5 months after irradiation.

This may also explain why no FDP were demonstrable in group II for some time though the patients must have had residual malignant tissue since later they had recurrences. Both the growth and the fibrinolytic activity of the tumours had presumably been extinguished temporarily because of injury to their vessels, especially of their adventitia. Not until the tumour had begun to recover with proliferating vessels did FDP recur in the serum. This explanation may hold also for the transient variation in the content of FDP after palliative radiation in group III.

Ascitic fluid often contains abundant fibrin. Albrechtsen et al (1) demonstrated the occurrence of plasminogen activators in malignant ascitic fluid. The occurrence, at the same time, of other proteolytic enzymes can, however, not be excluded. The occurrence of fibrinogen and fibrin in ascitic fluid together with the proteolytic activity might very well explain the high FDP values. The fall in FDP in ascitic fluid after radiotherapy may thus be ascribed to inhibition of the proteolytic activity of the cells.

We have earlier shown the diagnostic value of determination of FDP in cases with suspected ovarian tumours. This study shows that determination of FDP may also be valuable for checking the result of therapy and for detecting recurrences.

REFERENCES

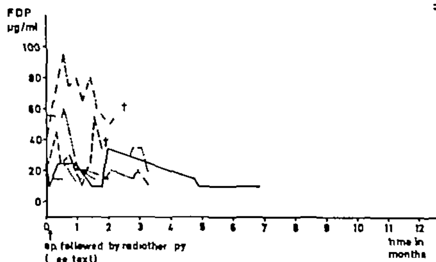
1. Albrechtsen, O. K., Storm, O. & Claassen, M. Fibrinolytic activity in some human body fluids. Scand J Clin Lab Invest *10* 310, 1958

10 732443 —

2. Astrup, T. Blood coagulation and fibrinolysis in tissue culture and tissue repair. Biochem Pharmacol, Suppl., p. 241. Pergamon, London, 1968.
3. Bykrka, G. Pandolfi, M. & Astedt, B. Release of fibrinolytic activators from human tumours cultured in vitro. Experientia *28*: 833 1972.
4. Böck, P. Kraus, H., Bittnel, G. & Koos, W. L'activité fibrinolytique des tumeurs cérébrales. Neuro-Chirurgie *16*. 542, 1970.
5. Clifton, E. E. & Grossi, C. E.: Fibrinolytic activity of human tumours as measured by the fibrin-plate method. Cancer *8*. 1146, 1955
6. Davidson, J. F. McNicol, G. P. Frank, G. L., Anderson, T. J. & Douglas, A. S. Plasminogen-activator-producing tumour. Brit Med J *1* 88, 1969
7. Errera, M. & Forssberg, A. G. Mechanisms in radiobiology. New York, Academic Press *2* 153 1960.
8. Hedner, U. & Nilsson, I. M. Clinical experience with determination of fibrinogen degradation products. Acta Med Scand *189*: 471 1971.
9. Laki, K. & Yancey, S. T. Fibrinogen (ed. Laki), p. 359. Marcel Dekker, New York, 1968.
10. Nagy, L., Kronmer, K., Gáti, I. & Keller, G. Die Untersuchung der Blutgerinnung bei der Strahlenbehandlung maligner gynäkologischer Tumoren. Z Geburtsh Gynäk *172*. 241 1970.
11. Nilehn, J.-E. Split products of fibrinogen after prolonged interaction with plasmin. Thrombos Diathes Haemorrh *18* 89 1967
12. Nilsson, I. M. & Pandolfi, M. Fibrinolytic response of the vascular wall. Thrombos Diathes Haemorrh, Suppl. *40* 231 1970
13. O'Meara, R. A. Q. Coagulative properties of cancers. Irish J Med Sci *394* 474 1958
14. Pandolfi, M. Studies on fibrinolysis in some tissues and in aqueous humour. Thesis. Berlingska Boktryckeriet, Lund, 1969
15. Peterson, H. I. Peterson, A. & Rudenstam, C.-M. Experimental studies on fibrinolysis in growth and spread of tumour. Acta Chir Scand, Suppl. 394 1968.
16. Santesson, L. Characteristics of epithelial mouse tumour cells in vitro and tumour structures in vivo. A comparative study. Acta Path Microbiol Scand, Suppl. 24, 1935
17. Thornes, R. D. Endogenous factors influencing host-tumor balance (ed. Wissler, Dao & Wood), p. 255. The University of Chicago Press, Chicago 1967
18. Todd, A. S. Histological localisation of fibrinolysin activator. J Path Bact *78* 281, 1959
19. Åstedt, B. Svanberg, L. & Nilsson, I. M. Fibrin degradation products and ovarian tumours. Brit Med J *4* 458, 1971
20. — Cancer, F D P and radiotherapy. Brit Med J 2. 47 1972.

Submitted for publication July 13 1972

Lars Svanberg
Coagulation Laboratory
Malmö allmänna sjukhus
Malmö
Sweden



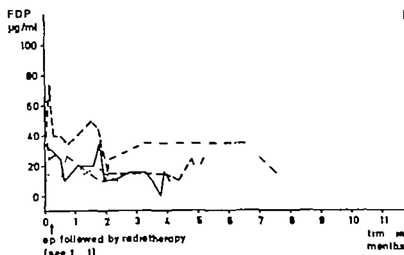


Fig 3

(9) analysed this clot-forming enzyme in tumour extracts and found that it was thrombin. They thought that a fibrin network is necessary for vascularisation and growth of tumours. It has long been known that malignant tissue can dissolve fibrin (2, 16) Clifton & Grossi (5) also showed that mesenchymal tumours exhibit a higher activity on fibrin plates than epithelial tissue. Fibrinolytic activity in malignant tissue has also been demonstrated histochemically (4 6 15)

FDP are demonstrable in blood from patients with generalised fibrinolysis, intravascular coagulation with secondary fibrinolysis or local fibrin deposits with secondary fibrinolysis Malignant tumours can cause such conditions because of their coagulative and fibrinolytic properties and give rise to FDP In a previous series (19) consisting of 163 patients with palpable ovarian masses we found FDP in the serum from 72 of those tumours which surgery revealed to be malignant.

In the present material FDP were demonstrated preoperatively in 84% An interesting observation made in the present investigation was that the concentration of FDP in the serum was influenced by radiotherapy

In all the 19 patients the FDP level first rose within 3–4 weeks after the beginning of radiotherapy This increase can probably be ascribed to destruction of tissue and vessels with the occurrence of thromboplastic substances and/or fibrinolytic enzymes in the bloodstream (10) There is no reason to ascribe this increase to the operation since Hedner & Nilsson (8) have shown that even after major operations only small amounts of FDP are demonstrable in the blood and then only during the first week after the operation.

The FDP then decreased or disappeared. Various explanations may be offered for this late decrease or disappearance of FDP For example

radiotherapy causes several degenerative changes in the irradiated tissue, and interferes especially with the enzyme processes in the cells. It has been shown histochemically (14, 18) that the fibrinolytic activity of the blood vessels in a tumour (the fibrinolytic activators are localised mainly to the vasa vasorum of the adventitia (12)) is related to its vascularity (2, 3). Irradiation of animals has been shown to produce changes in all layers of the vessel walls, but most in the adventitia because of its relatively high collagen content (7). Large doses result in hyalinisation of the collagen fibers. Even relatively small doses cause swelling of the endothelial cells and reduction of their ability to form new capillaries. The adventitia of the vessel walls has thus found to be missing in all animals killed 4–5 months after irradiation.

This may also explain why no FDP were demonstrable in group II for some time though the patients must have had residual malignant tissue since later they had recurrences. Both the growth and the fibrinolytic activity of the tumours had presumably been extinguished temporarily because of injury to their vessels, especially of their adventitia. Not until the tumour had begun to recover with proliferating vessels did FDP recur in the serum. This explanation may hold also for the transient variation in the content of FDP after palliative radiation in group III.

Ascitic fluid often contains abundant fibrin. Albrechtsen et al. (1) demonstrated the occurrence of plasminogen activators in malignant ascitic fluid. The occurrence, at the same time, of other proteolytic enzymes can, however, not be excluded. The occurrence of fibrinogen and fibrin in ascitic fluid together with the proteolytic activity might very well explain the high FDP values. The fall in FDP in ascitic fluid after radiotherapy may thus be ascribed to inhibition of the proteolytic activity of the cells.

We have earlier shown the diagnostic value of determination of FDP in cases with suspected ovarian tumours. This study shows that determination of FDP may also be valuable for checking the results of therapy and for detecting recurrences.

REFERENCES

1. Albrechtsen, O. K., Storm, O. & Claassen, M.: Fibrinolytic activity in some human body fluids. Scand J Clin Lab Invest *10* 310, 1958.
2. Astrup, T.: Blood coagulation and fibrinolysis in tissue culture and tissue repair. Biochem Pharmacol, Suppl., p. 241. Pergamon, London, 1968.
3. Björlin, G., Pandolfi, M. & Astedt, B.: Release of fibrinolytic activators from human tumours cultured in vitro. Experientia *28* 833, 1972.
4. Beck, F., Kraus, H., Wimel, G. & Koos, W.: L'activité fibrinolytique des tumeurs cérébrales. Neuro-Chirurgie *16* 542, 1970.
5. Clifton, E. E. & Grossi, C. E.: Fibrinolytic activity of human tumours as measured by the fibrin-plate method. Cancer *8* 1146, 1955.
6. Davidson, J. F., McNicol, G. P., Frank, G. L., Anderson, T. J. & Douglas, A. S.: Plasminogen-activator-producing tumour. Brit Med J *1* 88, 1969.
7. Ellinger, M. & Forssberg, A. G.: Mechanisms in radiobiology. New York, Academic Press 2. 153, 1960.
8. Hedner, U. & Nilsson, I. M.: Clinical experience with determination of fibrinogen degradation products. Acta Med Scand *189* 471, 1971.
9. Laki, K. & Yancey, S. T.: Fibrinogen (ed. Laki) p. 359. Marcel Dekker, New York, 1968.
10. Nagy, L., Krommer, K., Göltz, I. & Keller, G.: Die Untersuchung der Blutgerinnung bei der Strahlenbehandlung maligner gynäkologischer Tumoren. Z Geburtsh Gynäk *172* 241, 1970.
11. Niléhn, J.-E.: Split products of fibrinogen after prolonged interaction with plasmin. Thrombos Diathes Haemorrh *18* 89, 1967.
12. Nilsson, I. M. & Pandolfi, M.: Fibrinolytic response of the vascular wall. Thrombos Diathes Haemorrh, Suppl. 40: 231, 1970.
13. O'Meara, R. A. Q.: Coagulative properties of cancers. Irish J Med Sci *394* 474, 1958.
14. Pandolfi, M.: Studies on fibrinolysis in some tissues and in aqueous humour. Thesis. Berlingska Boktryckeriet, Lund, 1969.
15. Peterson, H. I., Peterson, A. & Rodenstam, C. M.: Experimental studies on fibrinolysis in growth and spread of tumour. Acta Chir Scand, Suppl. 394, 1968.
16. Santesson, L.: Characteristics of epithelial mouse tumour cells in vitro and tumour structures in vivo. A comparative study. Acta Path Microbiol Scand, Suppl. 24, 1935.
17. Thornes, R. D.: Endogenous factors influencing host-tumor balance (ed. Wissler, Dao & Wood), p. 255. The University of Chicago Press, Chicago, 1967.
18. Todd, A. S.: Histological localisation of fibrinolysin activator. J Path Bact *78* 281, 1959.
19. Astedt, B., Svanberg, L. & Nilsson, I. M.: Fibrin degradation products and ovarian tumours. Brit Med J *4* 458, 1971.
20. — Cancer F.D.P. and radiotherapy. Brit Med J 2. 47, 1972.

1   73/041

Submitted for publication Jul. 13, 1972

Lars Svanberg
Coagulation Laboratory
Malmö allmänna sjukhus
Malmö
Sweden

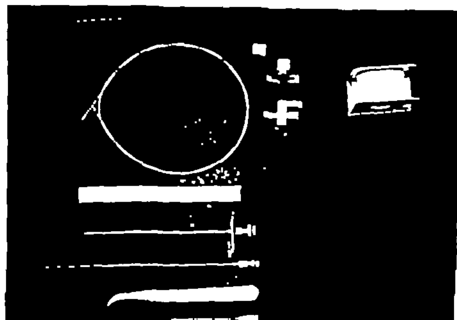


Fig 1

renal function. The intrapelvic pressure was also measured when the renal pelvis was punctured. A pressure abov 20 cm H_2O was regarded as pathological.



Fig 2 Wertheimoperated patient with a stricture of the left ureter The circleformed catheter is placed in the lower part of the renal pelvis.

RESULTS

The results are presented in Table III. The first group or permanent includes the patients with inoperable tumours of the pelvis. The second or temporary" group includes the remaining patients except for 2 in whom the pyelostomy was made only for diagnostic purposes and since in those cases the antegrade pyelography showed that no obstruction was present, the catheter was removed Table III shows the duration of the pyelostomy treatment and the effect on the renal function.

In the *Wertheim group* 2 patients had small ureterovaginal fistulae and moderate hydronephrosis. They were drained for 4–6 weeks and during that time repeated antegrade pyelography showed that the fistulae had healed and the catheter was then removed. Later urography and renogram showed normal conditions. Two patients

Table I *Material*

Diagnosis	Age	No
Cancer of the cerv op ad modum Wertheim	27–65	12
Inop tumours	31 74	11
Ureteral stones	36–71	10
Ureteral injuries	44–60	4
Cong hydronephrosis	21 39	3
Cancer of the urinary bladder	63–76	3
Others	46–53	2
		44

with more extensive ureterovaginal fistulae and a pronounced hydronephrosis were drained for 2 months, the ureters were then reimplanted into the urinary bladder. Both patients regained normal renal function. Four patients had extensive hydronephrosis due to severe strictures of the ureters. One of these cases was also complicated by ureteric stones. During drainage for 2–4 months *the hydronephrosis and renal function* was improved and the ureters could then be reconstructed with a part of the ileum in all 4 cases. In 2 patients the hydronephrosis was caused by a ecurrence of the cancer. After pyelostomy the renal function was maintained during radiotherapy treatment. In 2 cases with severe strictures, renal function was not improved during the pyelostomy treatment and as a split function test was very unsatisfactory on the affected side nephrectomy was undertaken.

In the group of patients with *ureteric stones* most of the patients were in a very bad condition with pain, impaired renal function and three of the patients had only one kidney due to a former nephrectomy. They were all considered as very bad risks for a general anaesthesia. During the drainage a dramatic improvement was noted. In three cases spontaneous passage of the stones occurred, the other cases were cured by surgery at the optimal time.

In the group of patients suffering from *inoperable tumours of the pelvis* a pronounced

Table II. *Indications for pyelostomy*

Groups of patient	No.	Hydronephrosis or impaired renal function: Urography	Renogram	Crh	Increased intrapelvic pressure
Wertheim					
(a) fistula	4	4	4	1	3
(b) stricture	8	8	8	3	8
Inop. tumours	11	11	11	10	8
Ureteral stones	10	10	7*	7	10
Ureteral injuries	3	3	3	1	2
Cancer of the bladder	3	3	3	3	3
Cong. hydronephrosis	3	3	2	2	1
Others	2	2	2	2	1
Total	44	43	40	29	36

* Only 8 patients examined.

Table III. *Result of the pyelostomy*

	No.	Duration (weeks)	Regress of hydronephrosis or improved renal function: Urography	Renogram	Crh
Pyelostomy permanent	11	3–31	11	11	10
Temporary	31	2–23	28	25	13
Diagnostic	2*				

* These cases are commented in the report of the result. In many other cases the pyelostomy added considerably to the diagnosis. In 4 cases split function (PAH clearance) was performed.

hydronephrosis was present on one or both sides. As treatment with radiotherapy or cytotoxic drugs was planned, *the pyelostomy was performed* to maintain the renal function during the period of treatment. In many cases the pyelostomy was kept for a long period of time, as Table III shows.

All the patients with *ureteric injuries* had fistulae urinary infection and hydronephrosis. Because of the difficulties of obtaining a retrograde pyelography antegrade pyelography was done and the catheter was left in order to drain the renal pelvis. One fist la closed during the pyelostomy treatment. One patient got improved renal function and a successful reimplantation could be done after 6 weeks drainage. The last patient was not improved and as the renal function was impaired due to a congenital hydronephrosis showing no improvement in spite of previous plastic surgery a nephrectomy was carried out.

Of the patients suffering from *cancer of the urinary bladder* two had severely impaired renal function when admitted to the hospital. After pyelostomy radiotherapy was started. The first patient died from his illness within a few weeks. The second improved with a satisfactory urine flow and decreasing creatinine in blood. A good regression of the tumour was noted *during the* radiotherapy and repeated antegrade pyelography showed regression of the hydronephrosis. Eight weeks after insertion, the pyelostomy catheter removed. Urogram and renogram almost returned to normal on the affected side. The last patient in this group is still undergoing radiotherapy treatment, the renal function is main-

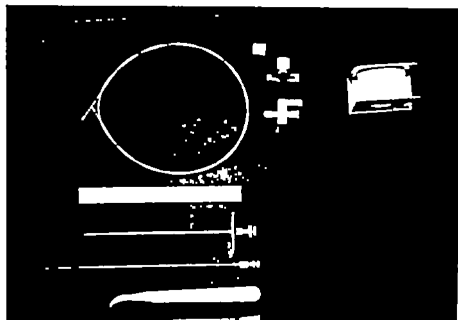


Fig 1

renal function. The intrapelvic pressure was also measured when the renal pelvis was punctured. A pressure above 20 cm H_2O was regarded as pathological.



Fig Wertheimoperated patient with a structure of the left ureter The circleformed catheter is placed in the lower part of the renal pelvis.

RESULTS

The results are presented in Table III. The first group or "permanent" includes the patients with inoperable tumours of the pelvis. The second or "temporary" group includes the remaining patients except for 2 in whom the pyelostomy was made only for diagnostic purposes and since in those cases the antegrade pyelography showed that no obstruction was present, the catheter was removed. Table III shows the duration of the pyelostomy treatment and the effect on the renal function

In the *Wertheim group* 2 patients had small ureterovaginal fistulae and moderate hydronephrosis. They were drained for 4–6 weeks and during that time repeated antegrade pyelography showed that the fistulae had healed and the catheter was then removed Later urography and renogram showed normal conditions. Two patients

Table I *Material*

Diagnosis	Age	No.
Cancer of the cervix op ad modum Wertheim	27–65	12
Inop tumours	31 74	11
Ureteral stones	36–71	10
Ureteral injuries	44–60	4
Cong hydronephrosis	21 39	3
Cancer of the urinary bladder	63–76	3
Others	46–53	2
		44

6. Fernström, I.: Personal communication, 1972.
7. Ekelund, L. Arteriovenous fistulae secondary to renal biopsy An experimental study in the rabbit. Acta Radiol *10*·218, 1970.
8. Gamam, H Zentralbl Gynäk *82*·1154, 1960.
9. Goodwin, W. E.: Percutaneous antegrade pyelography *I* Advances in diagnostic urology (ed. J J Kaufman) p. 44. Little Brown and Co Boston, 1964.
10. Goodwin, W E., Casey W C. & Woolf, W Percutaneous trocar (needle) nephrostomy in hydronephrosis. J Amer Med Ass *157* 891 1955
11. Hartl, H Zentralbl Gynäk *83* 1803 1964
12. Hohenfellner R. Die urologischen Komplikationen des Kollum-Karzinoms. Springer Verlag, 1965
13. Holmlund, D E. W Ureteral stones. Scand J Urol Nephrol, Suppl. 1, 1968.
14 Holmlund, D E. W & Löwegren, L. Renal angiography in ureteric obstruction. Scand J Urol Nephrol *5* 191, 1971
15 Liberu, H Renal angiography in experimental hydronephrosis Acta Radiol, Suppl. 136, 1956.
16 Jönsson, G Nord Medicin *65* 723 1961.
17 Kliver, O. & Tilli, F A. Deutsch Med Wschr *86*. 244 1961
18 Lingårdh, G Lundquist, B. & Lindström, B Renal arteriovenous fistula following puncture biopsy Scand J Urol Nephrol *5* 181 1971
19 Lundgren et al Acta obst gynec scand *31* 241 19??
20. Lundin, E. and Wadström, L. B. Translumbar pyelography Acta Chir Scand *130*·267 1965
21 Olsson, O.: Renal angiography *I* Angiography (ed. H. L. Abrams), vol. II, pp. 539–598. Little and Brown, Boston, 1961.
22. Seldinger, S. I.: Catheter-replace of the needle in percutaneous arteriography A new technique. Acta Radiol *39* 368, 1953.
23. Sotto, L. S Graham, J B. & Pickren, J W Am J Obst Gynec *80*·791, 1960.
24 Wecm, H. S. & Florence, T J The diagnosis of hydronephrosis by percutaneous renal puncture. J Urol *72*: 589 1954
25 Vela Navarrete, R Repeat direct pyelography via needle nephrostomy Acta Radiol *35* 1971
26. Wickbom, I Pyelography after direct puncture of renal pelvis. Acta Radiol *41* 505 1954
27 Widén, T Renal angiography during and after unilateral ureteric occlusion. Acta Radiol, Suppl. 162, 1958

Submitted for publication July 15 1972

Ulf Ulmsten
Department of Obstetrics and Gynecology
Malmö allmänna sjukhus
14 01 Malmö
S eden

Table IV *Complications*

Total no. of pyelostomy	Catheter slide	Haemorrhagia	Local infection
44	4	1	2

This case is commented on in the text report.

tained and the hydronephrosis has diminished at the time of this report.

Pending their admission for surgery three patients with *congenital hydronephrosis* had pyelostomy catheters for a period of 3–4 weeks. During this period the renal function was improved or maintained.

COMPLICATIONS

No serious side effects occurred. Table IV shows the complications. In 3 patients the catheter slid out of the renal pelvis, but reinsertion could easily be done in three cases. The fourth case was a patient with extremely extensive fibrosis after operation and radiotherapy treatment for cancer of the cervix uteri. She was drained because of an extensive stricture of the right ureter. The catheter slid out after 2 months but could easily be reinserted. When the catheter after 4 months of drainage once again slid out of the renal pelvis it was not possible to reinsert it and a minor haemorrhage occurred.

Repeated antegrade pyelography during the 4 months of pyelostomy treatment had shown no tendency to improvement. Plastic surgery was planned and ileoureterostomy was made. At the operation a haematoma around the renal pelvis was noted. Postoperatively a selective renal angiography showed no sign of arteriovenous fistula. In spite of that, the risk of making an a–v fistula cannot be completely disregarded (3, 6, 7, 18).

DISCUSSION

In cases of suspected postrenal obstruction it is important to get information about the site and the nature of the occlusion. It is also important, as soon as possible to drain the renal pelvis in order to maintain renal function until the optimal time for reconstructive surgery. Generally pyelostomy treatment should be done in these situations (16). Sometimes it can be difficult or impossible to get information about the obstruction by means of the conventional methods, i.e. retrograde pyelography. This was the case in many of our Wertheim patients due to extensive oedema of the bladderwall (caused by the trauma of the operation, the radiotherapy and simultaneous presence of urinary infection), or to kinking of the ureters after the operation, or to strictures with or without fistulae. The experience from the present series showed that percutaneous pyelostomy in such cases is of great value in combining a diagnostic and a therapeutic procedure. The patients with ureteric stones were all in a very bad condition which made it urgent in the acute situation to drain the renal pelvis via the diagnostic catheter. In cases where the diagnosis is made by conventional methods and the patient is in good condition, pyelostomy may be done by conventional surgery. The passage of the stones, which occurred in three cases, was obviously facilitated by the decrease of pressure above the stones (13).

In the patients with inoperable tumours of the pelvis we made the pyelostomy while other therapy was planned. In cases where no therapy was possible we hesitated to do a pyelostomy as it might just lengthen the patients suffering. In general the percutaneous pyelostomy should only be used for short periods though many of our patients could be drained for long periods, as is evident from Table III.

Thus, percutaneous pyelostomy should be seriously considered an alternative method for both diagnostic and therapeutic use in all cases where conventional methods for some reason are unsuccessful or doubtful.

REFERENCES

1. Altvater, G. & Imholz, G. Geburtsh Frauenheilk 20: 1 14 1960.
2. Bartley, O., Chidekel, N. & Rådberg, C. Percutaneous drainage of renal pelvis for uremia due to obstructed urinary outflow. Acta Chir Scand 129: 443, 1965.
3. Boijsen, E. & Kohler, R. Renal arteriovenous fistulae. Acta Radiol 57: 433, 196..
4. Casey, W. C. & Goodwin, W. E. Percutaneous antegrade pyelography and hydronephrosis. J Urol 74: 164, 1955.
5. Edholm, P., Fernstrom, I., Lindblom, K. & Seldinger, S. I. Roentgen television in practice with special regards to puncture examinations. Acta Radiol, Suppl. 16, 196..

6. Fernström, J. Personal communication, 1972.
7. Ekelund, L. Arteriovenous fistulae secondary to renal biopsy. An experimental study in the rabbit. Acta Radiol *10*: 218, 1970.
8. Gassner, H. Zentralbl Gynäk *82*: 1154, 1960.
9. Goodwin, W. E. Percutaneous antegrade pyelography. *In* Advances in diagnostic urology (ed. J. J. Kaufman), p. 44. Little, Brown and Co., Boston, 1964.
10. Goodwin, W. E., Casey, W. C. & Woolf, W. Percutaneous trocar (needle) nephrostomy in hydronephrosis. J Amer Med Ass *157*: 891, 1955.
11. Hartl, H. Zentralbl Gynäk *86*: 1603, 1964.
12. Hohenfellner, R. Die urologischen Komplikationen des Kollum-Karzinoms. Springer-Verlag, 1965.
13. Holmlund, D. E. W.: Ureteral stones. Scand J Urol Nephrol, Suppl. 1, 1968.
14. Holmlund, D. E. W. & Lövegren, L.: Renal angiography in ureteric obstruction. Scand J Urol Nephrol *5*: 193, 1971.
15. Idbohrn, H. Renal angiography in experimental hydronephrosis. Acta Radiol, Suppl. 136, 1956.
16. Larsson, G. Nord Medicin *65*: 723, 1961.
17. Klietz, O. & Illig, F. A. Deutsch Med Wschr *86*: 465, 1961.
18. Lindbom, G., Lindqvist, B. & Lindström, B. Renal arteriovenous fistula following puncture biopsy. Scand J Urol Nephrol *5*: 181, 1971.
19. Lundgren et al. Acta obst gynec scand *51*: 241, 1972.
20. Lundin, E. and Wadström, L. B. Translumbar pyelography. Acta Chir Scand *130*: 267, 1965.
21. Olsson, O. Renal angiography. *In* Angiography (ed. H. L. Abrams), vol. II, pp. 539–598. Little and Brown, Boston, 1961.
22. Seldinger, S. L. Catheter-replace of the needle in percutaneous arteriography. A new technique. Acta Radiol *39*: 368, 1953.
23. Sotto, L. S., Graham, J. B. & Pickren, J. W. Am J Obst Gynec *80*: 791, 1960.
24. Weens, H. S. & Florence, T. J. The diagnosis of hydronephrosis by percutaneous renal puncture. J Urol *72*: 589, 1954.
25. Vela Navarrete, R. Repeat direct pyelography via needle nephrostomy. Acta Radiol *11*, 1971.
26. Wickbom, I. Pyelography after direct puncture of renal pelvis. Acta Radiol *41*: 505, 1954.
27. Widén, T. Renal angiography during and after unilateral ureteric occlusion. Acta Radiol, Suppl. 162, 1958.

Submitted for publication July 15, 1972

Ulf Ulmsten
Department of Obstetrics and Gynecology
Malmö allmänna sjukhus
214 01 Malmö
Sweden

Acta Obstet Gynec Scand 52, 153–156, 1973

DIAZEPAM (VALIUM®) AS AN ANAESTHETIC FOR OPERATIVE VAGINAL DELIVERY

Norvald Sagen and Kjell Haram

From the Department of Obstetrics and Gynecology (Head: Professor Oddmund Koller), The University of Bergen, Bergen, Norway

Abstract. In a clinical controlled trial the use of vinyl-ether-ether N_2O (53 cases) and diazepam-N_2O (48 cases) as general anaesthetic for operative vaginal delivery is compared. Local anaesthetic for episiotomy was applied in both methods to enhance analgesia. In the diazepam group there were no maternal complications, while in the vinyl-ether-ether group there were 14 cases of vomiting, of which 2 led to aspiration with development of Mendelson's syndrome. A third case in the same group also had an aspiration without evident sequelae. The time from start of anaesthetic induction till complete delivery of the baby was on average 6 min 17 sec in the vinyl-ether-ether group compared with 2 min 21 sec in the diazepam group, which means an average reduction of about 4 min. Diazepam anaesthesia is simple to administer, effective, allows rapid delivery and is comfortable and safe for the mother. This form of anaesthesia made the obstetrician's task easier during delivery. It also appears to be a safe method for the newborn, judged by the Apgar scoring system.

Diazepam has been employed for some years to alleviate pain and tension during the first stage of labour. A number of investigators have come to the conclusion that there are negligible effects on the newborn (1, 2, 12, 8). Diazepam has also been used for the induction of anaesthesia for laparotomy, caesarean section and minor surgical procedures (16, 17, 13, 19). Diazepam alone or in combination with local anaesthesia of the perineum, has been used as a sedative during forceps delivery and for suture of the episiotomy (9, 14, 15). Vinylether (Vinydan®)-ether N_2O anaesthesia, or ether alone, have been used for many years for forceps and breech deliveries. These methods have been considered simple and safe for mother and child. However, maternal vomiting is common both during induction and recovery from this form of anaesthesia. Occasionally other complications arise, such as aspiration of gastric contents into the bronchial passages, which leads to further complications (5).

In a clinically controlled trial we compared diazepam-N_2O anaesthesia with Vinydan-ether N_2O anaesthesia. Local anaesthesia for episiotomy was given in both groups. In this preliminary report we are concerned most with the anaesthetic effect on the mother and with the obstetrical conditions for operative delivery. The final aim is to clarify the effects on the newborn.

MATERIAL AND METHODS

This series includes 104 consecutive operative vaginal deliveries, 69 were forceps deliveries and 35 were breech deliveries. A total of 101 anaesthetics were given—there were 3 sets of twins.

There were fetal and maternal indications for these operative deliveries. Rotation forceps deliveries are included. We do not use high forceps and very seldom outlet forceps. Assisted breech deliveries are usual but breech extraction is performed when there are signs of intrauterine asphyxia and for the delivery of the second twin. Løvset's method of shoulder delivery is used routinely. Firstly the delivery of the head is attempted using the Guillemeau

Table 1

Diazepam-group	Vinydan-ether group
Xylocain-Exadrin 0.5 % infiltrated into perineum (20 ml)	Xylocain-Exadrin 0.5 % infiltrated into perineum (20 ml)
Premedication: Atropine 0.6 mg I.V.	Premedication: Atropine 0.6 mg I.V.
Diazepam 30 mg, rapid intravenous injection (30 sec)	Vinydan-ether
N_2O : O_2 (75 % : 25 %) given at rate 8 l/min	N_2O : O_2 (75 % : 25 %) given at rate 8 l/min

Table V. *Maternal complications caused by the general anaesthesia*

Type of anaesthetic	Complications: Vomiting	Long-term excitation	Aspiration of vomit	Total no. of complications	Total no. of patients
Diazepam	0	0	0	0	48
Vinydan-ether	14	4	3	21	53

tocography (Table VII). The cephalic and breech presentations are not separately tabulated.

DISCUSSION

Anaesthesia for operative vaginal deliveries is an important problem. From a study of 579 maternal deaths in England and Wales in 1964–65 50 were shown to be associated with complications from anaesthesia and at least 52% of these deaths were due to aspiration of stomach contents (5).

During general anaesthesia, vomiting and regurgitation of acidic stomach contents with aspiration into the bronchi can lead to pneumonia. The danger can be reduced by giving alkali (magnesium trisilicate) to make the stomach contents less acidic (18). Nourishment during labour is necessary but creates problems since emptying of the stomach is delayed: even those receiving intravenous feeding cannot be regarded as fasting if they have eaten after contractions commence (6).

In our study three patients in the Vinydan-ether group had complications in the form of aspiration of stomach contents. Two contracted Mendelsohn's syndrome (Table V). There was also a marked tendency to vomiting and maternal discomfort during induction and recovery (Table II). Diazepam anaesthesia is superior since there were no cases of vomiting, even in those patients who had eaten just before receiving anaesthesia (Table V). All the mothers were satisfied with diazepam anaesthesia (Table II). All had amnesia for the period from induction of anaesthesia until recovery—a phenomenon which appears typical of diazepam anaesthesia (4, 11).

Diazepam anaesthesia creates good delivery conditions for the obstetrician (Table III). Induction is rapid and allows fast delivery even in those cases where the anaesthesia was classified as unsatisfactory (Table IV). This is probably because of diazepam's muscle-relaxant properties (7, 10). Intravenous diazepam has little effect on respiration and circulation (7).

The dose of 30 mg diazepam is not necessarily optimal for each patient since response can vary with bodyweight and medication before and during labour.

A study has shown that diazepam given in an intravenous dose of 20 mg, 5 min before delivery had no ill effect on the newborn (9).

We do not think that the dose of diazepam which was employed in this study has untoward effects on the newborn but our numbers are as yet too small to allow us to come to a definite conclusion regarding this. However, we have the

Table VI. *Newborn having had no signs of intra-uterine asphyxia*

Type of anaesthetic	Time (min)	Number of newborn: Apgar score 1–4	Apgar score 5–6	Apgar score 7–10	Total
Diazepam	1	1	1	19	21
	5			21	21
Vinydan-ether	1	1	3	4	[illegible]
	5		1	27	28

Table VII. *Newborn having had signs of intra-uterine asphyxia*

Type of anaesthetic	Time (min)	Number of newborn: Apgar score 1–4	Apgar score 5–6	Apgar score 7–10	Total
Diazepam	1	12	5	12	29
	5	2	5	22	29
Vinydan-ether	1	6	7	13	26
	5		5	21	26

Table II *Maternal opinion on anaesthesia*

Type of anaesthetic	Number of mothers				
	Effective	Comfortable	Ineffective	Uncomfortable	Total
Diazepam	48	48	0	0	48
Vinydan-ether	47	15	6	38	53

technique ("big bow") At any sign of resistance to delivery of the head by this method, Pipers forceps are applied and the head is gently eased over the perineum.

When required the mothers got pethidine and diazepam during the first stage of labour

Patients were assigned to each group by a system of random selection. The maternal age range was 16–41 years in the diazepam group and 16–4 years in the Vinydan-ether group, the average age being 25 years in each group.

In order to reduce the demand for basal anaesthetic we added N_2O on mask and infiltrated a local anaesthetic for episiotomy

The two anaesthetic methods are shown in Table I. As can be seen they are identical apart from the main anaesthetic agent.

It should be noted that 30 mg diazepam are dissolved in 9 ml physiological saline This gives a fine white suspension which must not be stored (information from Hoffman-La Roche).

The general anaesthetics were given by an anaesthetic nurse

Evaluation of the methods was done both by the mother and by the obstetrician. Assessment of the condition of the newborn was made by the Apgar score. Acid-base estimations in the new-born were also done. Because the numbers in this preliminary study are small and there are many variable factors, acid-base values have not yet been correlated with clinical states.

Table III *Obstetrician s judgement of anaesthesia*

Type of anaesthetic	Number of patients			
	Good	Satisfactory	Unsatisfactory	Total
Diazepam	34	11	3	48
Vinydan-ether	24	16	13	53

Table IV *Time from commencement of anaesthetic induction till clamping of umbilical cord*

Type of anaesthetic	Mean	No.	Range
Diazepam	2 1	46	1 10 – 5
V nydan-ether	6 17	52	– 20

RESULTS

1 *Opinion on anaesthesia*

The *maternal* opinion on the anaesthesia was classified as *effective* which means no pain *comfortable* which means no untoward symptoms during induction and recovery The designations *ineffective* and *uncomfortable* were the opposites of the two above (see Table II) The mothers gave their opinion after they were fully conscious.

The *obstetrician s* assessment of the anaesthesia was based on the degree to which restlessness was present during delivery *good* the patient was quiet *satisfactory* there was a slight restlessness, *unsatisfactory* she was so restless as to make delivery disturbed. (See Table III)

2. *Delivery time*

Three pairs of twins, two in the Valium®-group and one in the Vinydan® group are not included. (See Table IV)

With the diazepam method we gained almost 4 min Breech and forceps deliveries took about the same length of time.

3 *Maternal complications*

These were limited here to those observed during hospital stay which is 5 days in an uncomplicated case. The complications are described in Table V Aspiration means that aspiration of stomach contents have resulted in laryngospasm, respiratory difficulties and cyanosis.

4 *Evaluation of the status of the newborn*

This was made according to the Apgar scale at 1 and 5 min after delivery 1–4 means serious asphyxia 5–6 mild asphyxia and 7–10 normal score The babies were divided into two groups—those with no signs of intrauterine asphyxia (Table VI) and those with signs of intrauterine asphyxia, as judged either clinically or by cardio-

Acta Obstet Gynec Scand 51 157–160 1973

DETECTION OF THE "PREGNANCY ZONE" PROTEIN BY MEANS OF AN IMMUNODIFFUSION METHOD

Lars Beckman, Bo von Schoultz and Torgny Stigbrand

From the Division of Medical Genetics, Department of Clinical Bacteriology (Head: Prof. Jan Carlsson), Obstetrics and Gynaecology (Head: Prof. Per Lundström) and Medical Chemistry (Head: Prof. K.-G. Paul), University of Umeå, Umeå, Sweden

Abstract. A precipitating monospecific rabbit antiserum was produced by immunization with purified "pregnancy zone" protein. By means of double diffusion in agar gel 27 out of 30 pregnant women at term and 23 out of 30 women treated with oral contraceptives were found to have the "pregnancy zone" protein in their serum. The "pregnancy zone" protein was not detectable in 30 sera from non-pregnant women, 30 cord sera and 30 samples of amniotic fluid. A comparison between the electrophoretic and immunodiffusion methods for the detection of the "pregnancy zone" protein showed that the immunodiffusion method was about twice as sensitive as the electrophoretic method.

The "pregnancy zone" protein is a human α_2 serum globulin (2) found in pregnancy (1, 2, 3, 6, 8, 11, 15) and after administration of oral contraceptive drugs (4, 7, 11). The function of the "pregnancy zone" protein is not known so far but it has been suggested that it may be a specific protein carrier of oestrogen, progesterone and their metabolites (1, 2). In general the presence or absence of the "pregnancy zone" protein at term seems to have no detectable influence on fetal welfare (2, 6), but it has been suggested that the incidence of congenital anomalies may be increased among babies of women lacking the "pregnancy zone" protein (2).

In previous studies the detection of the "pregnancy zone" protein has been made by means of electrophoresis in starch- or polyacrylamide gels. There are two main difficulties involved in the electrophoretic method for recording the "pregnancy zone" protein. 1) Unless the electrophoretic conditions are rigidly standardized the "pregnancy zone" may sometimes coincide in its mobility with certain haptoglobin bands and thus the haptoglobin type may influence the detectability (6, 8, 14). 2) In sera with a low concentration of the "pregnancy zone" protein it may not be possible to visualize a distinct protein band.

It would clearly be advantageous to develop a technique for the detection of the "pregnancy zone" protein which is more sensitive and which does not rely on electrophoretic separation, e.g. an immunological method. This paper describes the production of a specific rabbit antibody against purified "pregnancy zone" protein (14) and the application of this antibody in studies of the "pregnancy zone" protein by means of double diffusion in agar gel.

MATERIAL AND METHODS

Immunization

"Pregnancy zone" protein was purified according to the procedure described by von Schoultz & Stigbrand (14). In order to increase the yield of the preparations to obtain sufficient amounts of protein for immunization the criteria for purity were kept less rigid so that some contamination by high molecular serum proteins was allowed. One rabbit weighing approximately 3 kg was immunized with a solution of purified "pregnancy zone" protein from one pregnant woman. The protein solution which contained about 1 mg of protein in 2 ml phosphate buffer pH 7.4 was mixed with one volume of complete Freund's adjuvant and given as multiple subcutaneous injections at two different occasions with eight days interval. A sample of the solution of purified protein was saved for future immunological experiments. Blood was collected from the marginal vein of the rabbits ear 7 days after the last injection and kept overnight at 4°C. Serum was then removed, centrifuged free of formed elements and stored at −25

impression that the results are not less favourable for the newborn than in the control group (Tables VI and VII). We believe that diazepam anaesthesia has advantages in cases of intrauterine asphyxia since it allows rapid delivery such that the total period of asphyxia can be shortened. Our results are so encouraging that the study is continuing.

ACKNOWLEDGEMENT

We express our gratitude to Mr Kjell Gunnarshaug for his valuable assistance and to Hoffmann-La Roche for technical support.

REFERENCES

1 Bepko F Lowe E & Waxman, B. Relief of the emotional factor in labor with parenterally administered diazepam. Obstet Gynecol *26* 852, 6, 1965
2. Berger M Die Anwendung von 7-Chlor 1,3-dihydro-1 methyl-5-phenyl-2H 1 4-benzodiazepin-2 (diazepam) bei 3 000 Gebärenden. Arzneim-Forsch (Drug res.) *16* 1110, 1966.
3 Bonica J J Obstetric Analgesia and Anaesthesia. I, pp. 401–413 Blackwell, Oxford, 1967
4. Brown, S. S & Dundee J W Clinical studies of induction agents XXV Diazepam. Brit J Anaesth *40* 108, 1968.
5 Crawford, J S. The anaesthetist's contribution to maternal mortality Brit J Anaesth *42* 70, 1970
6. Davison, J S., Davison, M. C & Hay D M Gastric emptying time in late pregnancy and labour J Obstet Gynaec Brit Comm *77* 37 1970
7 Dundee J W & Keilty S. R., Diazepam Internat Anaesth Clin 7 1 91 1969
8. Flowers, C. E., Rudolph, A. J & Desmond, M M Diazepam (Valium) as an adjunct in obstetric analgesia. Obstet Gynec *34* 1 68 1969
9 German, L. J Basal sedation with intravenous Diazepam for instrumental vaginal deliveries. Third Internat. Congr., London, 1971. Psychosom Med Obstet Gynaec (ed. N Morris, London), pp. 44–47
10. Hüter J., Über Wirksamkeit, Nebenwirkungen, Indikationen und Kontraindikationen von Diazepam (Valium) in der Geburtshilfe und Frauenheilkunde. Klin Wschr *13* 681 1968.
11 McClish, A., Diazepam as an intravenous induction agent for general anaesthesia. Canad Anaesth Soc *13* 562, 1966.
1_ Niswander K. R., Effect of diazepam on meperidine requirements of patients during labor Obstet Gynec *34* 1 62, 1969
13 Rouchy R., Blodeau, P Le Cannellier R., Greze J. & Grosleux, P Le diazepam par voie intra-veineuse en obstétrique. *Presse Med 5 7 312, 1966.*
14 Serment, H., Teyssier G. & Guillet, F Utilisation du Valium intraveineux en obstétrique Provence Med *37* 8–9 43 1969
15 Stoerl, P R. & Bossart, H. Sédation centrale par le Valium intraveineux pour la suture de l'épisiotomie, Gynaecologia (Basel) *167* 324 1969
16. Stovner J & Endresen, R., Diazepam in intravenous anaesthesia. Lancet 1298 1965
17 Stovner J & Endresen, R., Intravenous anaesthesia with Diazepam. Acta Anaesth Scand *4* 223, 1966.
18 Williams, M Crawford, J S Titration of magnesium tricilicate mixture against gastric acid secretion. Brit J Anaesth *43* 783 1971
19 Zehntner H., Schaub, W & Ott, R., Erfahrungen mit Valium in der Kleinchirurgie. Ther Gegenw *110* 515 1971

Submitted for publication Aug 7 1972

Norvald Sagen
Department of Obstetrics and Gynaecology
School f Medicine
University f Bergen
5000 Bergen
Norw y

Acta Obstet Gynec Scand 51 157–160 1973

DETECTION OF THE "PREGNANCY ZONE" PROTEIN BY MEANS OF AN IMMUNODIFFUSION METHOD

Lars Beckman, Bo von Schoultz and Torgny Stigbrand

From the Division of Medical Genetics, Department of Clinical Bacteriology (Head: Prof. Jan Carlsson), Obstetrics and Gynaecology (Head: Prof. Per Lundström) and Medical Chemistry (Head: Prof. K.-G. Paul), University of Umeå, Umeå, Sweden

Abstract. A precipitating monospecific rabbit antiserum was produced by immunization with purified "pregnancy zone" protein. By means of double diffusion in agar gel 27 out of 30 pregnant women at term and 23 out of 30 women treated with oral contraceptives were found to have the "pregnancy zone" protein in their serum. The "pregnancy zone" protein was not detectable in 30 sera from non-pregnant women, 30 cord sera and 30 samples of amniotic fluid. A comparison between the electrophoretic and immunodiffusion methods for the detection of the "pregnancy zone" protein showed that the immunodiffusion method was about twice as sensitive as the electrophoretic method.

The "pregnancy zone" protein is a human α_2 serum globulin (2) found in pregnancy (1, 2, 3, 6, 8, 11, 15) and after administration of oral contraceptive drugs (4, 7, 11). The function of the "pregnancy zone" protein is not known so far but it has been suggested that it may be a specific protein carrier of oestrogen, progesterone and their metabolites (1, 2). In general the presence or absence of the "pregnancy zone" protein at term seems to have no detectable influence on fetal welfare (2, 6), but it has been suggested that the incidence of congenital anomalies may be increased among babies of women lacking the "pregnancy zone" protein (2).

In previous studies the detection of the "pregnancy zone" protein has been made by means of electrophoresis in starch- or polyacrylamide gels. There are two main difficulties involved in the electrophoretic method for recording the "pregnancy zone" protein. 1) Unless the electrophoretic conditions are rigidly standardized the "pregnancy zone" may sometimes coincide in its mobility with certain haptoglobin bands and thus the haptoglobin type may influence the detectability (6, 8, 14). 2) In sera with a low concentration of the "pregnancy zone" protein it may not be possible to visualize a distinct protein band.

It would clearly be advantageous to develop a technique for the detection of the "pregnancy zone" protein which is more sensitive and which does not rely on electrophoretic separation, e.g. an immunological method. This paper describes the production of a specific rabbit antibody against purified "pregnancy zone" protein (14) and the application of this antibody in studies of the "pregnancy zone" protein by means of double diffusion in agar gel.

MATERIAL AND METHODS

Immunization

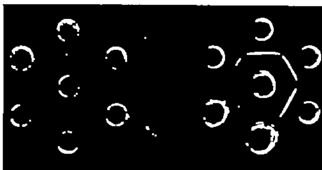
"Pregnancy zone" protein was purified according to the procedure described by von Schoultz & Stigbrand (14). In order to increase the yield of the preparations to obtain sufficient amounts of protein for immunization the criteria for purity were kept less rigid so that some contamination by high molecular serum proteins was allowed. One rabbit weighing approximately 3 kg was immunized with a solution of purified "pregnancy zone" protein from one pregnant woman. The protein solution, which contained about 1 mg of protein in 2 ml phosphate buffer pH 7.4, was mixed with one volume of complete Freunds adjuvant and given as multiple subcutaneous injections at two different occasions with eight days interval. A sample of the solution of purified protein was saved for future immunological experiments. Blood was collected from the marginal vein of the rabbit ear 7 days after the last injection and kept overnight at 4°C. Serum was then removed, centrifuged free of formed elements and stored at −25°.

Double diffusion tests

Double diffusion experiments were performed according to Ouchterlony (12) on glass slides with 2 mm thick layers of 1% agarose (Behring-Werke) in 0.1 M sodium phosphate buffer pH 7.4. Six peripheral and one central well were cut in the agar. The diameter of the wells was 3 mm and the distances between wells were 5 mm. The rabbit immune serum was placed in the central well and the human sera to be tested in the peripheral wells. The wells were filled with 10 µl of serum and the glass slides were kept in a moist chamber at 37°C. The precipitates were examined against a dark background by oblique illumination from below after 1 and 3 days.

Electrophoresis

After addition of haemoglobin the serum samples were examined by means of starch gel electrophoresis with the buffer system by Poulik (13). Some samples were also examined by means of polyacrylamide electrophoresis with 7% polyacrylamide and the buffer system by Ashton & Braden (5). Staining was performed with Amidoblack 10 B.



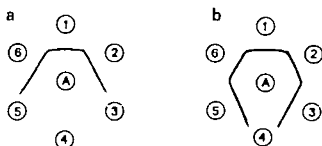


Fig. 1. Precipitation reactions in agar gel between rabbit antiserum (A) in central wells and purified "pregnancy zone" protein, sera and amniotic fluid in the peripheral wells. Upper part, photograph; lower part, schematic drawing. To the left (*a*) in the peripheral wells: 1 purified "pregnancy zone" protein, 2, serum from woman taking oral contraceptives, 3 serum from non-pregnant woman, 4 amniotic fluid, 5 cord serum, 6, serum from pregnant woman at term. To the right (*b*) in the peripheral wells. 1 purified "pregnancy zone" protein and 5 serum from women taking oral contraceptives, 3 and 6, serum from pregnant women at term. 4 serum from non-pregnant women.

Table I. *Comparison of the electrophoretic (Elf) and immunodiffusion (Imm) methods for the detection of the "pregnancy zone" protein*

(+) and (−) indicate presence and absence of "pregnancy zone" protein respectively

	Imm(+) Elf (+)	Imm(+) Elf (−)	Imm(−) Elf (+)	Imm(−) Elf (−)	
Pregnant women at term	14	13	0	3	30
Women taking contraceptives	13	10	0	7	30
Non-pregnant women	0	0	0	30	30
Cord sera	0	0	0	30	30
Amniotic fluid	0	0	0	30	30
	27	23		100	150

Sera and amniotic fluids

Thirty serum samples each were examined from pregnant women at term, non-pregnant women, women taking oral contraceptives and newborn infants (cord serum). (These samples were selected at random from material previously investigated (6, 7).) In addition thirty different samples of amniotic fluids from different stages of pregnancy were tested.

RESULTS

When the rabbit antiserum was tested against the solution of purified "pregnancy zone" protein one strong precipitation line was found. In addition there were two weak precipitates closer to the central well. After absorption of the rabbit serum with serum from a healthy human male only the major precipitation line remained. The monospecific precipitin thus obtained was found to react also with some sera from pregnant women at term and from women taking oral contraceptives. A reaction of identity was found between these two types of sera and between the sera and the purified "pregnancy zone" protein (Fig. 1).

Serum samples and amniotic fluids were tested by means of the immunodiffusion method (Table I). Out of 30 pregnant women at term 27 showed a positive reaction with the immunodiffusion method while with the electrophoretic method 14 had been classified as having the "pregnancy zone". The corresponding figures for 30 women taking oral contraceptives were 23 and 13 respectively. Thus out of 50 cases which were positive with the immunodiffusion method

27 (54%) showed a detectable pregnancy zone on starch gel electrophoresis. The sera from non-pregnant women, the cord sera and the amniotic fluids were negative with both methods.

DISCUSSION

The purification of the "pregnancy zone" protein has made it possible to produce a specific rabbit antiserum against this protein and to develop an immunodiffusion method for its detection. The immunodiffusion method was found to be about twice as sensitive as the electrophoretic method used by us. The frequency of detectable "pregnancy zone" protein among pregnant women has been quite variable in previous investigations (2, 3, 6, 8, 15), most likely depending on technical factors.

In a previous investigation Hirschfeld & Söderberg (9) using immunoelectrophoresis found proteins in the sera of pregnant women which were not detectable in non-pregnant subjects. One of these proteins may be identical with the "pregnancy zone" protein. The specificity of the immunological method makes it preferable to zone electrophoresis for identification purposes. With the electrophoretic method protein zones other than the "pregnancy zone" but with a similar migration rate could be wrongly classified as the "pregnancy zone". Other types of error could arise for example from individual electrophoretic variation of the "pregnancy zone" protein or from its conversion to smaller protein fragments in storage. Such variations would lead to underestimates of the occurrence of the "pregnancy zone" protein with the electrophoretic method, but most likely not with the immunological method.

The present results provide conclusive evidence that the extra electrophoretic zones seen in pregnancy and after administration of oral contraceptives represent the same protein. Already the previous results suggested with a high degree of probability that this was the case, but the same mobility on unidimensional electrophoresis was no final proof of molecular identity.

The higher sensitivity of the immunological method is also of importance in the assessment of the absence of the "pregnancy zone" protein. Thus in 50 non-pregnant women, cord sera and amniotic fluid there was no trace of this protein. This could mean that the protein is confined to the maternal circulation and that it appears when induced by certain steroids. Perhaps the most interesting finding is the fact that some pregnant women at term and some women taking oral contraceptives do not develop the "pregnancy zone" protein. From Fig. 1 (*a*, sample 3 and *b*, sample 4) it can be seen that the serum samples showing a negative reaction have very little if any "pregnancy zone" protein. Thus there were no "bending reactions" of the precipitation lines of the neighbouring wells. It seems that the induction of the "pregnancy zone" protein is a normal phenomenon in pregnancy and during administration of oral contraceptives, but that some women lack the ability to produce this protein. In future investigations it would e.g. be of interest to study whether this inability to produce the "pregnancy zone" protein is under genetic control, and whether the inability to produce this protein during pregnancy is correlated to the inability to produce it during administration of oral contraceptives.

The sera from non-pregnant women, who were all negative, have been stored for 1 year and have been frozen and thawed 3 times. The sera from pregnant women at term have been stored for 3 years and have been frozen and thawed 6 times. The fact that 90% of these sera showed a detectable "pregnancy zone" indicates that this protein is very stable.

The immunological reagent against the "pregnancy zone" protein should be a valuable tool in future quantitative studies by means of radial immunodiffusion according to Mancini et al. (10). Such studies are under way.

ACKNOWLEDGEMENTS

This investigation was supported by grant from the Swedish Medical Research Council and the Medical Faculty of the University of Umeå. We thank Dr L. E. Jonsson, Uppsala, for putting the samples of amniotic fluid at our disposal.

REFERENCES

1. Alonso, J. F. & de Alvarez, R. R. Amer J Obstet Gynec 84: 815, 1963.
2. Alonso, J. F. Amer J Obstet Gynec 89: 204, 1964.
3. Alonso, J. F. & Tarahim, N. G. Amer J Obstet Gynec 84: 199, 196_.

4 de Alvarez, R. R. & Afonso, J. F. Penn Med J 70 43 1967
5 Ashton, G. C. & Braden, A. W. H. Aust J biol Sci 14 48 1961
6 Beckman, L., Beckman, G. & Stigbrand, T. Hum Hered 20 530, 1970.
7 Beckman, L., von Schoultz, B. & Stigbrand, T. Acta Obstet Gynec Scand 50, 369 1971
8. Cooper D. W., Nature 200 892, 1963
9 Hirschfeld, J & Söderberg, U Nature 187 33., 1960
10. Mancini, G Vaerman, J P Carbonara, A. O & Heremans, J F *In* Proteides of the Biological Fluids (ed. H Peeters). Proceedings of the XI Colloquium Bruges 1963, Amsterdam, 1964 p. 370.
11 Margolis, J & Kendrick, K. G. Aust J exp Biol med Sci 47 637 1969
12. Ouchterlony Ö Acta Path Microbiol Scand 25 186, 1948.
13 Poulik, M D Nature 180: 1477 1957
14 von Schoultz, B. & Stigbrand, T Acta Obstet Gynec Scand 51 51 1972.
15 Smithies, O. Advances Protein Chem 14 65 1959

Submitted for publication Aug 8, 197

Bo von Schoultz
Department of Obstetrics and Gynaecology
University
901 85 Umeå 6
Sweden

Acta Obstet Gynec Scand 52. 161–165 1973

A METHOD OF MEASURING THE INTERSPINOUS DIAMETER BY AN ULTRASONIC TECHNIQUE

A Preliminary Report

Vlasta Václavínková

From the Department of Obstetrics and Gynecology (Head. Professor Axel Ingelman-Sundberg), Sabbatsberg Hospital, Karolinska Institutet Stockholm, Sweden

Abstract A method was developed of measuring the interspinous diameter of the female pelvis with the ultrasound B-system Vidoson 635. This method was used in a group of 30 patients, who simultaneously had X-ray pelvimetry. The difference between the results obtained by ultrasonic measurement and X-ray was $\Delta M = \pm 0.13$ cm. Other authors have achieved similar differences by ultrasonic measurement of the biparietal diameter of the fetal head and the sagittal diameter of the pelvic brim.

At present the routine method of measuring the pelvic diameters is by X-ray. Even if the dose is minimized, and the examinations postponed to the end of pregnancy there may be a certain risk for the fetus. According to English investigations (25, 26) the risk of developing malignant tumours, especially in the central nervous system, seems to be increased during infancy even after a single X-ray examination during pregnancy.

Ultrasonic energy at least in doses used for obstetrical examinations, on the other hand, seems to be harmless to the fetus (1, 7, 9, 13, 19, 21).

Several authors (3, 4, 5, 6, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 20, 21, 22, 23) have used ultrasonic pelvimetry for the measurement of the diameters of the pelvic inlet and the biparietal diameter of the fetal head. As far as we have been able to find from the literature however nobody has published results of measuring the pelvic outlet with an ultrasonic technique.

These diameters are of major importance since contraction of the pelvic outlet nowadays is a bigger problem than contraction of the inlet which mostly can be diagnosed clinically.

TECHNIQUE

A Vidoson 635 which is an ultrasonic unit for ultrasonic diagnoses using the B-scan technique has been used. With the aid of pulsed ultrasound (US), sectional displays of echoes from biological tissues are produced on the screen of a cathode ray tube (dimensions 9.3 × 10 cm). The Vidoson 635 uses an ultrasonic frequency of 2.5 MHz with pulse repetition of 2 KHz. The maximum ultrasonic intensity is 10 mW cm² The screen image can be photographed with a Polaroid camera. The Vidoson is mounted on a mobile stand and the probe moves easily in all directions (24).

EXPERIMENTS WITH MODEL

With a skeleton pelvis in water we looked for the most convenient angle and position for application of the ultrasonic probe and at the same time we made several scans of pelvic sections for our orientation. Then we found what we considered to be the most convenient way to put the probe on to the outlet of the pelvis in order to receive the best echo from the ischial spines. In this position the spines are very close to the probe and, from a practical point of view we received the best contact (27).

Fig. 1 shows schematically the geometrical application of the probe in a B in different pelvic sections. The scans can be seen in Fig. 2. The probe was moved from sacrum to symphysis. Fig. 2 a shows caudal part of sacrum. By pushing the probe under control across the distal part of the pelvis we approached the most narrow point, the interspinous diameter (Fig. 2 b). In Fig. 2 a plastic cup has been placed in the pelvis to represent the fetal



Fig. 1. Geometrical application of the probe in all as different pelvic sections (ex. 1).

4. de Alvarez, R. R. & Afonso, J. F. Penn Med J *70* 43 1967
5. Ashton, G. C. & Braden, A. W. H., Aust J biol Sci *14* 248 1961
6. Beckman, L., Beckman, G. & Stigbrand, T., Hum Hered *20* 530, 1970.
7. Beckman, L., von Schoultz, B. & Stigbrand, T., Acta Obstet Gynec Scand *50* 369 1971
8. Cooper D W., Nature *200* 892, 1963
9. Hirschfeld, J & Söderberg, U Nature *187* 332, 1960.
10. Mancini, G., Vaerman, J P Carbonara, A. O & Heremans, J F *In* Proteides of the Biological Fluids (ed. H Peeters). Proceedings of the XI Colloquium Bruges 1963 Amsterdam, 1964 p. 370.
11. Margolis, J & Kendrick, K. G., Aust J exp Biol med Sci *47* 637 1969
12. Ouchterlony Ö Acta Path Microbiol Scand 25 186, 1948
13. Poulik, M D Nature *180* 1477 1957
14. von Schoultz, B. & Stigbrand, T Acta Obstet Gynec Scand *51* 51 1972.
15. Smithies, O Advances Protein Chem *14* 65 1959

Submitted for publication Aug 8, 1972

Bo von Schoultz
Department of Obstetrics and Gynaecology
University
901 85 Umeå 6
Sweden

A METHOD OF MEASURING THE INTERSPINOUS DIAMETER BY AN ULTRASONIC TECHNIQUE

A Preliminary Report

Vlasta Václavinková

From the Department of Obstetrics and Gynaecology (Head. Professor Axel Ingelman-Sundberg), Sabbatsberg Hospital, Karolinska Institutet, Stockholm, Sweden

Abstract A method was developed of measuring the interspinous diameter of the female pelvis with the ultrasound B apparatus Vidoson 635. The method was used in a group of 30 patients, who simultaneously had X-ray pelvimetry. The difference between the results obtained by ultrasonic measurement and X-ray was $\Delta d \sim \pm 0.13$ cm. Other authors have achieved similar differences by ultrasound measurement of the biparietal diameter of the fetal head and the sagittal diameter of the pelvic brim.

At present the routine method of measuring the pelvic diameters is by X-ray. Even if the dose is minimized, and the examinations postponed to the end of pregnancy there may be a certain risk for the fetus. According to English investigations (25, 26) the risk of developing malignant tumours, especially in the central nervous system, seems to be increased during infancy even after a single X-ray examination during pregnancy.

Ultrasonic energy, at least in doses used for obstetrical examinations, on the other hand, seems to be harmless to the fetus (1, 7, 9, 13, 19, 21, 23).

Several authors (3, 4, 5, 6, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 20, 21, 22, 23) have used ultrasonic pelvimetry for the measurement of the diameters of the pelvic inlet and the biparietal diameter of the fetal head. As far as we have been able to find from the literature however, nobody has published results of measuring the pelvic outlet with an ultrasonic technique.

These diameters are of major importance, since contraction of the pelvic outlet nowadays is a bigger problem than contraction of the inlet which usually can be diagnosed clinically.

TECHNIQUE

A Vidoson 635 which is an ultrasonic unit for ultrasonic diagnosis using the B-scan technique has been used. With the aid of pulsed ultrasound (US), sectional displays of echos from biological tissues are produced on the screen of a cathode ray tube (dimensions 9.3 × 10 cm). The Vidoson 635 uses an ultrasonic frequency of 2.5 MHz with pulse repetition of 2 KHz. The maximum ultrasonic intensity is 10 mW/cm². The screen image can be photographed with a Polaroid camera. The Vidoson is mounted on a mobile stand and the probe moves easily in all directions (24).

EXPERIMENTS WITH MODEL

With a skeleton pelvis in water we looked for the most convenient angle and position for application of the ultrasonic probe and at the same time we made several scans of pelvic sections for our orientation. There we found what we considered to be the most convenient way to put the probe on to the outlet of the pelvis in order to receive the best echo from the ischial spines. In this position the spines are very close to the probe and, from a practical point of view, we received the best contact (27).

Fig. 1 shows schematically the geometrical application of the probe as well as different pelvic sections. The scans can be seen in Fig. 2. The probe was moved from sacrum to symphysis. Fig. 2 a shows caudal part of sacrum. By pushing the probe under control across the distal part of the pelvis we approached the most narrow point, the interspinous diameter (Fig. 2 b). In Fig. 2 a plastic cup has been placed in the pelvis to represent the fetal



Fig. 1. Geometrical application of the probe as well as different pelvic sections (ex. I).

Fig. 2. US echos of different pelvic sections (application ex. I). (a) Caudal part of sacrum, section 1. (b) Interspinous diameter section 2. (c) Same section as in Fig. 2b with a plastic cup to represent the fetal head. (d) Interspinous diameter with "fetal head" section 3.

head. (Same sections as in Fig. 2b.) Pushing the probe further one reaches a place where the distance is again increasing and the intertuberous diameter is almost reached (Fig. 2d). Fig. 3 shows another angle. Again different pelvic sections are shown in Fig. 4.

After many experiments we found that the angle at which the probe was applied was of no importance as the spines have peaked ends, and therefore the ultrasound can reflect perpendicularly and come back to the probe in different positions and angles. The most convenient position has to be determined for each patient individually.



Fig. 3. Geometrical application of the probe as in II at different pelvic sections (ex. II).

CLINICAL MEASUREMENTS

On the basis of the above experience we started a clinical investigation. For practical reasons we placed the patient in lithotomy position. For reasons of hygiene and control of infection, a polyethylene sheet was placed on the probe, the sheet being smeared on both sides. The sheet was changed between every investigation and it must be carefully applied to avoid air bubbles. This protection was of no hindrance during the investigation. We examined a group of women who had been X-rayed during the last month of pregnancy because a narrow pelvis was suspected. Thus we could compare our results with the X-ray examinations. The echograms are simple to interpret.

Fig. 5 shows the contact surface between probe and patient and two points where the medial measurement indicates the desired line. When calculating it is very important to use the lowest amplitude, i.e. when the points are nearly disappearing, otherwise the thickness of the points could cause an incorrect measurement.

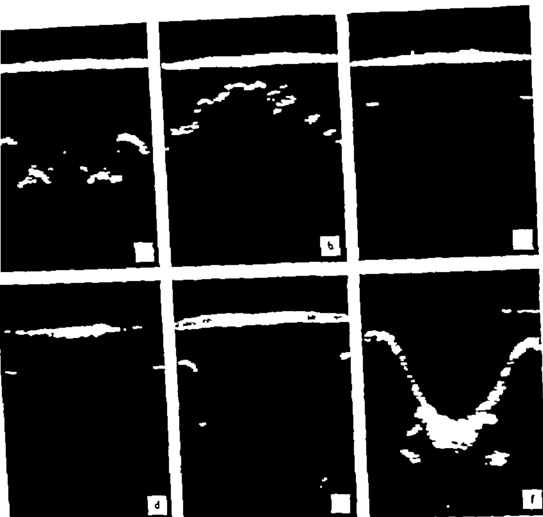


Fig 4 US echos of different pelvic sections (applications vs. US) (*a*) Part of posterior iliac spines and sacrum, section 1 (*b*) Major part of sacrum, section 2. (*c*) Interspinous diameter section 3 (*d*) Interspinous diameter same section 3, but in patient. ( ) Ischial tuberosity section 4 (*f*) Sub-pubic arch, section 5

Tab 1 shows the results. A comparison of the US results show the small differences in the two measurements, the average difference (Δd) being within the limits of error of the two methods.

DISCUSSION

The results of US investigations do not differ significantly from the results achieved by X-ray pelvimetry and they are within the limits of error of the methods. Similar conclusions have been obtained also by authors studying biparietal diameter of the fetus and the sagittal diameter of the pelvic brim. It is unlikely that the different position of the apparatus during X-ray and during US investigation could affect the comparability of the results obtained by the two different methods. In the same way it is improbable that the sacrospinous ligament could disturb the investigation. It is obvious that the personal experience of the examiner is important in the critical examination of an echogram.

This report is a preliminary one and we hope that, on the basis of wider experience of examining a large number of patients, the method described will lead the way to further possibilities of using US in obstetric diagnosis.

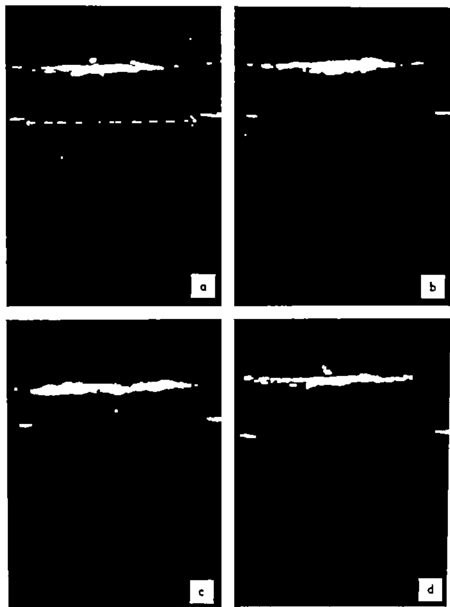


Fig 5 a, b c d. Practical illustrations (examples) of interspinous diameter in patients.

ACKNOWLEDGEMENT

My sincere thanks to Ing. Lundgren and Elema-Schonander AB for kind provision of equipment.

REFERENCES

1 Boyd, E., Abdula, U Doland, L & Flemming, J E. E. Chromosome breakage nd ultrasound. Brit Med J 501 1971
2 Fernström, I Bäckenmätning. Svensk Forening för Medicinsk Radiologi, Stockholm, 1968.
3 Garrett, W J & Robinson, D E. Assessment of fetal size and growth rate by ultrasonic echoscopy Obst and Gyn 38 525 1971
4 Hansmann, M Nellen, J & Henning, H. Klinische Ergebnisse einer modifizierten Methodik der Ultraschalldiagnostik in der Geburtshilfe Arch Gynäk 11 264 1971
5 Hinselmann, M Ultraschalldiagnostik in der Geburtshilfe Deutsch Med Wschr 94 953 No. 18/1969.
6. H Ilander H. J Anwendungsmöglichkeiten der Ultraschalldiagnostik in der Geburtshilfe und Frauenheilkunde Wien Med Wschr 120: 602 No. 36 1970.
7 Johannigmann, J Zahn, V Thieme, R. & Kloe B. J Der Einfluss des Ultraschalles auf den intrauterinen Druck. Z Geburtsh Gynäk 174 14 1971
8 Kohorn, E. L, Morrison, J Ashford, C & Blackwell, R. J Ultrasonic scanning in obstetrics and gynecology Obstet Gynec 34 515 1969
9 Kratochwil, A Ultraschalldiagnostik in Geburtshilfe und Gynäkologie Georg Thieme Verlag, Stuttgart, 1968
10 — Ultraschalldiagnostik in der Geburtshilfe und Gynäkologie Zbl Gynak 91 113 1969
11 — Die Ultraschalldiagnostik in der Geburtshilf Med Klin 64 1573 No. 36 1969
1 — Die Bedeutung der Ultraschalldiagnostik für Ge-

Table I. *Comparing X-ray* (cm) *and US* (cm) *results of the measurement of interspinous diameter*

Patient	X-ray	US	Δcm
1	10.5	10.5	0
2	10.5	10.4	−0.1
3	10.5	10.6	+0.1
4	9.9	9.8	−0.1
5	10.2	10.0	0.2
6	11.6	11.6	0.0
7	11.6	11.6	0.0
8	9.8	10.3	+0.5
9	9.7	9.8	+0.1
10	10.2	10.3	+0.1
11	10.0	9.9	−0.1
12	10.7	10.7	0.0
13	9.5	9.2	−0.3
14	10.3	9.9	−0.4
15	9.8	10.0	+0.2
16	8.3	8.3	0.0
17	10.8	10.8	0.0
18	11.6	11.4	−0.2
19	9.5	9.6	+0.1
20	9.1	9.2	0.1
21	10.8	11.3	0.5
22	10.5	10.5	0.0
23	10.5	10.7	+0.2
24	10.9	10.9	0.0
25	11.8	11.7	0.1
26	10.5	10.5	0.0
27	9.8	9.9	0.1
28	10.2	10.1	−0.1
29	11.6	11.7	+0.1
30	9.9	10.0	0.1

Δd 0.13 cm.

burtshilfe und Gynäkologie. Schweiz Med Wschr 99 992, 1969.

13. — Schadigungsmöglichkeiten durch Ultraschalldiagnostik. Gynäk Rdsch 9 240, 1970.

14. Krause, W. & Soldner, R.: Ultraschallbildverfahren (B-scan) mit hoher Bildfrequenz für medizinische Diagnostik. Electromedica 4 1967 (Sonderdruck).

15. Levi, S. & Flament, P. L'ultrasonopelvimétrie. Note liminaire à propos de 25 cas. Bull Soc Roy Belge Gyn Obst 39 355, 1969.

16. Levi, S., Ultrasonic diagnosis in obstetrics: The clinical value of measuring the biparietal diameter of fetus. Gynec Obstet 69 227 1970.

17. Loch, E. G.: Erste Ergebnisse in der Ultraschalldiagnostik. Electromedica 4 1969 (Sonderdruck).

18. Loch, E. G. & Strathmann, J. Die Messgenauigkeit des Ultraschalls im Vergleich zu Röntgenaufnahmen des Beckens. Strahlentherapie 139 459 1970.

19. Loch, E. G.: Ultraschall in der Schwangerenberatung. Arch Gynäk 211 268, 1971.

20. Machelett, R. & Deal, R.: Möglichkeiten und Ergebnisse der Ultraschall-Diagnostik in der Geburtshilfe. Zbl Gynäk 93 81 1971.

21. Mosler, K. H. Teichert, F. Scheuer H. & Mitschka, F. Ultraschallüberwachung in der Präventivgeburtshilf. Med Klin 65 1250, No. 26/1970.

22. Rezacova, Z., Bergner, J. & Voegeli, H. Bilan des examens par ultrasons à la Policlinique universitaire de Gynécologie et d'Obstétrique de Genève. Bull Fed Soc Gynec Obstet Franc 22 288 1970.

23. Rosenov N. Frischkorn, R. & A Castano y Almendral. Untersuchungen zur Ultraschall-Diagnostik in Gynäkologie und Geburtshilfe. Strahlentherapie 136: 458, 1968.

24. Siemens Aktiengesellschaft: Ultraschall-Bildgerät VI DOSON 635.

25. Stewart, A. M. & Kneale G. W. Age-distribution of cancers caused by obstetric X-ray and their relevance to cancer latent periods. Lancet II. 4 No. 7662/1970.

26. — Radiation dose effects in relation to obstetric X rays and childhood cancers. Lancet I 1185 No. 7658 1970.

27. Váchavinková, V. Mätning av interspinalt avstånd med ultraljud. Gyn. kongress, Åland, 1971.

Submitted for publication Aug. 8, 1972

Vlasta Váchavinková
Department of Obstetrics and Gynaecology
Sabbatsberg Hospital
113 82 Stockholm
Sweden

Acta Obstet Gynec Scand 52, 167–170 1973

THE TRANSFER OF DIAZEPAM ACROSS THE PLACENTA DURING LABOUR

R. Erkkola, L. Kangas and A. Pekkarinen

From the Department of Obstetrics and Gynaecology (Head. Professor L. Rauramo) and Department of Pharmacology (Head. Professor A. Pekkarinen) / Turku University Turku, Finland

Abstract The transfer of diazepam through the placenta during labour was studied by the gas chromatographic method developed by us using Ni^{63}-electron capture detector 10 mg of diazepam was given intramuscularly to 37 patients during the first stage of labour 5–401 min before delivery after which blood samples were immediately collected from the umbilical cord and the maternal vein. Diazepam was found in all the plasma samples with very great individual variation in the concentrations. In one particular case, in which the delivery took place 5 minutes after the diazepam injection, the corresponding diazepam concentrations in the cord plasma and in the maternal plasma were 506 ng/ml and 504 ng/ml. In 31 cord plasma samples collected 26–401 min after the diazepam injection the mean diazepam concentration was 79 ng/ml, and in the maternal plasma was 39 ng/ml. The average foetal/maternal concentration ratio during the same time period was 2.0 and in the whole series 1.8. Neither the diazepam concentrations in the cord plasma nor the foetal/maternal ratios of the concentrations had any significant influence on the Apgar scores of the newborn. The cause of the diazepam accumulation in the foetal circulation is unclear. The better binding of diazepam with the foetal plasma proteins is discussed. The importance of individual drug doses to parturients according to their weight is emphasized.

Diazepam is widely used to induce sedation and muscular relaxation in mothers during the first and second stages of labour (5, 6, 11, 14). Nevertheless little study has been done on the transfer of diazepam across the placenta and the influence of different diazepam concentrations on the newborn. This study was thus undertaken, especially as diazepam is quite commonly used in our own obstetrical department.

Passive diffusion according to Fick's Law is considered the most important means of placental transfer of drugs (10, 1., 16). Passive diffusion is nowadays understood to mean the penetration of an unbound drug through the placenta according to the concentration gradient. Being a lipid-soluble undissociated drug with a small molecular weight diazepam easily penetrates the biological membranes. Thus earlier studies showed that diazepam easily diffuses through the placenta, both in man and in animals. Cavanagh & Condo (2) gave 10 mg of diazepam intramuscularly to six mothers during labour and in their gas chromatographic study observed that the maximum concentration of diazepam in the maternal plasma was 0.17 µg/ml, and in the cord plasma 0.16 µg/ml. The mean diazepam concentration ratio between the maternal and foetal plasma was 0.89. DeSilva et al. (3) in their study observed the ratio of maternal/foetal concentrations to be 1.11 on average. Diazepam was found in the foetal blood 4 minutes after an intravenous injection of diazepam. Idanpään-Heikkilä et al. (7) found that after an intramuscular injection C^{14} labelled diazepam crossed the placenta quickly in early human pregnancy and the diazepam concentration of the cord blood was approximately double that in the maternal blood even 6 hours after the injection. In another study Idanpään-Heikkilä et al. (8) observed that C^{14}-labelled diazepam crosses the placenta of monkeys, hamsters and rats.

MATERIAL AND METHOD

During the first stage of labour 10 mg of diazepam (Tensopam® Lääke Oy) was administered to 37 mothers in cases when labour was expected to be normal. The average age of the women was 24.8 years. The deliveries took place 5–401 min after the injection and blood samples were collected in heparinized test tubes simultane-

Acta Obstet Gynec Scand 52, 167–170, 1973

THE TRANSFER OF DIAZEPAM ACROSS THE PLACENTA DURING LABOUR

R. Erkkola, L. Kangas and A. Pekkarinen

From the Department of Obstetrics and Gynaecology (Head: Professor L. Kaarano) and Department of Pharmacology (Head: Professor A. Pekkarinen) of Turku University Turku, Finland

Abstract. The transfer of diazepam through the placenta during labour was studied by the gas chromatographic method developed by us using Ni^{63}-electron capture detector. 10 mg of diazepam was given intramuscularly to 37 patients during the first stage of labour 5–401 min before delivery, after which blood samples were immediately collected from the umbilical cord and the maternal vein. Diazepam was found in all the plasma samples with very great individual variation in the concentrations. In one particular case, in which the delivery took place 5 minutes after the diazepam injection, the corresponding diazepam concentrations in the cord plasma and in the maternal plasma were 406 ng/ml and 504 ng/ml. In 31 cord plasma samples collected 26–401 min after the diazepam injection the mean diazepam concentration was 38 ng/ml, and in the maternal plasma was 18 ng/ml. The average foetal/maternal concentration ratio during the same time period was 2.0 and in the whole series 1.8. Neither the diazepam concentrations in the cord plasma nor the foetal/maternal ratios of the concentrations had any significant influence on the Apgar scores of the newborn. The cause of the diazepam accumulation in the foetal circulation is unclear. The better binding of diazepam with the foetal plasma proteins is discussed. The importance of individual drug doses to parturients according to their weight is emphasized.

Diazepam is widely used to induce sedation and muscular relaxation in mothers during the first and second stages of labour (5 6 11 14). Nevertheless little study has been done on the transfer of diazepam across the placenta and the influence of different diazepam concentrations on the newborn. This study was thus undertaken, especially as diazepam is quite commonly used in our own obstetrical department.

Passive diffusion according to Fick's Law is considered the most important means of placental transfer of drugs (10 12, 16). Passive diffusion is [illegible] the penetration of an unbound drug through the placenta according to the concentration gradient. Being a lipid-soluble undissociated drug with a small molecular weight diazepam easily penetrates the biological membranes. Thus earlier studies showed that diazepam easily diffuses through the placenta, both in man and in animals. Cavanagh & Condo (2) gave 10 mg of diazepam intramuscularly to six mothers during labour and in their gas chromatographic study observed that the maximum concentration of diazepam in the maternal plasma was 0.17 µg/ml, and in the cord plasma 0.16 µg/ml. The mean diazepam concentration ratio between the maternal and foetal plasma was 0.89. DeSilva et al. (3) in their study observed the ratio of maternal/foetal concentrations to be 1.11 on average. Diazepam was found in the foetal blood 4 minutes after an intravenous injection of diazepam. Idänpään-Heikkilä et al. (7) found that after an intramuscular injection C^{14}-labelled diazepam crossed the placenta quickly in early human pregnancy and the diazepam concentration of the cord blood was approximately double that in the maternal blood even 6 hours after the injection. In another study Idänpään-Heikkilä et al. (8) observed that C^{14}-labelled diazepam crosses the placenta of monkeys, hamsters and rats.

MATERIAL AND METHOD

During the first stage of labour 10 mg of diazepam (Tensopam® Lääke Oy) was administered to 37 mothers in cases where labour was expected to be normal. The average age of the women was 24.8 years. The deliveries took place 5–401 min after the injection and blood samples were collected in heparinized test tubes simultane-

Table I *Distribution of patients according to the interval between the diazepam injection and blood sampling*

Group	Interval (minutes)	No. of patients
1	0–15	5
2	16–45	4
3	46–75	5
4	76–105	5
5	106–135	5
6	136–165	5
7	166–195	4
8	196–401	4
Total		37

ously from the cubital vein of the mother and from the umbilical cord.

Plasma was separated by centrifugation and the samples stored at −20°C until analyzed. The diazepam concentration in plasma remains unchanged, in our experience for at least months. Diazepam concentrations in plasma were analyzed by gas chromatography using a Ni[63] electron capture detector according to the modification of two earlier methods (9 13).

A plasma sample of 0.5 ml was first made slightly alkaline with 50 mg of K_2HPO (anhydrous) and extracted twice with 3.0 ml of diethyl ether Waterphase was separated and the combined diethyl ether extracts were dried at 45°C in a water bath by an air stream. Water was then completely removed from the dried extract in a vacuum desiccator over silicogel overnight. The dried, waterfree residue of diethyl ether extract was diluted in 0.5–2.0 ml of *n*-hexane and a 0.3 µl aliquot from the dilution was injected into the gas chromatograph. The recovery of diazepam standard, added to plasma, with this method, is 94.3% A Varian Model 2100 was used as the gas chromatograph and Varian 20 as the recorder The glass columns were U-tubes (5 feet long and 2 mm in inner diameter) These tubes were filled with Chromosorb Q (100–120 mesh) covered with 3 SE 30 (Varian) as liquid phase The temperature of the injector was 280°C and the temperature of the oven was

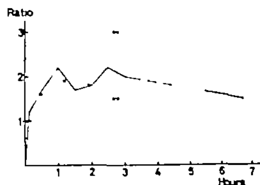


Fig 1 The individual cord level, maternal level ratios of diazepam concentrations and the mean ratios in different time groups.

programmed to rise by 4°C/min from 220°C to 260 C Nitrogen (AGA 0.005) was used as a carrier gas with a flow rate of 32 ml/min and a pressure of 4 0 kp cm² A Ni[63] detector (Varian) was used as the electron capture detector The Ni[63]-detector temperature was 360°C and the voltage 10–20 mV The diazepam of the plasma samples was standardized by external diazepam standards. The cord and maternal plasmas were analyzed under the same conditions each time successively and double determinations were made on all diazepam plasma samples. The precision of the double determinations of the diazepam in the plasma samples was 0.4 %.

RESULTS

The grouping of the cases by the time interval between the diazepam injection and blood sampling and the number of case pairs in each group are shown in Table I

Diazepam was found in all the plasma samples. The diazepam concentrations in the umbilical cord and maternal vein are shown in Table II The highest diazepam concentration was found in the case in which the blood samples were taken only 5 min after the diazepam injection In this case the diazepam concentration in the cord plasma was as high as 506 ng/ml and in the maternal plasma 504 ng/ml In the samples taken 26–401 min (31 case pairs) after the diazepam injection the mean diazepam concentration in the cord plasma was 70 ng/ml ± 13 ng/ml (S E M) and in the maternal plasma 38 ng/ml ± 6 ng/ml (S E M). The difference is highly significant ($p < 0.001$) In the case in which the blood samples were not received until 401 min after the diazepam injection the diazepam concentration in the cord plasma was 64 ng/ml, and 44 ng ml in the maternal plasma. The individual diazepam concentration ratios (foetal plasma/

Table II *The mean concentrations of diazepam in maternal and cord plasma* (±S E.M)

Group	Cord level (ng/ml)	Maternal level (ng/ml)
1	163 ± 89	151 ± 90
2	68 ± 32	51 ± 4
3	58 ± 31	30 ± 15
4	96 ± 45	47 ± 18
5	91 ± 54	53 ± 31
6	52 ± 4	21 ± 7
7	74 ± 21	43 ± 13
8	58 ± 10	34 ± 6

maternal plasma) are shown in Fig. 1. In the earliest sample pair taken 5 min after the diazepam injection, the concentration ratio was 1.0. After that the ratio increased, and in the samples taken at 26–401 min the mean foetal/maternal ratio of the diazepam concentrations was 2.0. In the whole material it is 1.8.

The mean score for all infants is Apgar 8.5 at 1 min and 8.9 at 15 min. These are not different from the Apgar scores of an adequate control series. The mean Apgar scores in those ten cases in which the diazepam concentration was more than 100 ng/ml, were 8.2 and 9.0. In the cases, in which the diazepam concentration in the cord plasma was below 100 ng/ml, the Apgar scores were 8.7 and 9.6. The differences in the corresponding Apgar scores are not significant. Neither did we notice any somnolence or limpness in the new-born.

DISCUSSION

Our study lends strong support to earlier observations, according to which diazepam very rapidly crosses the human placenta. Five minutes after the intramuscular injection diazepam was already found in the foetal circulation in the same very high concentration as in the mother. Besides this our study confirms the very interesting finding made by Cavanagh & Condo (2) at the time of labour and by Idänpään-Heikkilä et al. (7) in early pregnancy that diazepam accumulates in the foetal circulation.

More than 95% of the diazepam in the plasma is bound to plasma proteins (15). In our method we measured the concentration of total diazepam, but the amounts of free and unbound diazepam have unfortunately not been examined separately. If the transfer of diazepam across the placenta occurs by passive diffusion, the amount of free diazepam should be the same in the maternal and foetal circulations. If this is so, the main reason for the accumulation of diazepam in the foetal blood would be the better binding of diazepam to the foetal plasma proteins. According to the study made by Ehrnebo et al. (4) the binding of many drugs (for example fenemal) with foetal plasma proteins is lower than with adult plasma proteins.

In the opinion of Idänpään-Heikkilä et al. (7) the better binding properties of foetal plasma proteins or red cells were the reason for the higher concentration of diazepam in the foetal blood. According to our study the better binding with the foetal plasma is sufficient to explain the accumulation. Beckett & Taylor (1) considered the acidity of the foetal blood as the main reason for the accumulation of pethidine in the foetal blood. This hardly explains the accumulation of diazepam, which is an undissociated drug.

The mean Apgar score for all infants is 8.5 at centrations at the time of birth after a routine dose of 10 mg of diazepam are a very noteworthy reminder to the clinician. This great variation is partly the result of weight differences in the parturients, but the main reasons are the difference in the absorption from the injection site, the distribution to the tissues and the metabolism and elimination of diazepam. According to van der Kleijn (15) the most important cause of different individual concentrations is the high accumulation of diazepam in fat tissues. The only thing the clinician can do is to give diazepam and all other drugs so that the doses are in relation to the patient's weight. It is nevertheless impossible to predict the actual diazepam concentration in the parturient after one intramuscular injection of diazepam.

In our study we noticed no statistically significant correlation between the diazepam concentrations in the foetal circulation and the Apgar scores. Flowers et al. (5) have noticed some limpness in newborns but no changes in the Apgar scores after 40–50 mg of diazepam given to the parturient during labour. The explanation of the very good tolerance to two-threefold concentrations in the cord plasma compared with the maternal plasma may be that the concentration in the cerebral circulation is not so high. Idänpään-Heikkilä et al. (8) have noticed the accumulation of diazepam in foetal liver tissue in animals.

REFERENCES

1. Beckett, A. H. & Taylor, J. F. Blood concentrations of pethidine and pentazocine in mother and infant at the time of birth. J Pharm Pharmacol *19*: 503, 1967.
2. Cavanagh, D. & Condo, D. R. Diazepam—a pilot study of drug concentrations in maternal blood, amniotic fluid and cord blood. Curr Ther Res 6: 122, 1964.
3. DeSilva, J. A. F., D'Arconte, L. & Kaplan, J. The

determination of blood levels and the placental transfer of diazepam in humans. Curr Ther Res *6* 115 1964
4. Ernebo, E., Agurell, S. Jalling, B. & Borens, L. O. Age differences in drug binding by plasma proteins. Studies on human foetuses, neonates and adults. Eur J Clin Pharmacol *3* 169 1971
5. Flowers, C. E., Rudolph, A. J. & Murdina, M. M.. Diazepam (Valium) as an adjunct in obstetrical analgesia. Obstet Gynec *34* 68 1969
6. Friedman, E. A., Niswander, K. R. & Sachtleben, M. R.. Effect of diazepam on labour. Obstet Gynec *34* 82, 1969
7. Idänpään-Heikkilä, J. E., Jouppila, P. I. Puolakka, J. O. & Verne, M. S. Placental transfer and fetal metabolism of diazepam in early human pregnancy. Am J Obstet Gynecol *109* 1011 1971
8. Idänpään-Heikkilä, J. E., Taska, R. J. Allen, H. A. & Schoolar, J. G. Placental transfer of diazepam-C in mice hamsters and monkeys. J Pharmacol Exp Ther *176* 752, 1971
9. Marcucci, F. Fanelli, R. & Mussini, E.. A method for gas chromatographic determination of benzodiazepines. J Chromatogr *37* 318, 1968.
10. Moya, F. & Smith, B. E.. Placental transport of drugs and anesthetics. Anesthesiology *26*, 465 1965.
11. Nisbet, R., Boulas, S. H. & Kantor, H. I.. Diazepam (Valium) during labor. Obstet Gynec *29* 726, 1967
12. Page, E. W.. Transfer of materials across the human placenta. Am J Obstet Gynecol *74* 705 1957
13. Schubert, B. & Malmgren, G.. Diazepam—en klinisk analytisk undersökning. Opuscula Med *8*, *38* 1969
14. Timonen, S. & Hagner, M. Benzodiazepines in an unselected obstetrical series. Ann Chir Gynaecol Fenn *55* 65 1966.
15. van der Kleijn, E.. Kinetics of distribution and metabolism of diazepam in animals and humans. Arch Int Pharmacodyn Ther *182* 433, 1969
16. Villee, C. A.. Placental transfer of drugs. Ann NY Acad Sci *123* 237 1965

Submitted for publication Aug 19 197

Risto Erkkola
Department of Obstetrics and Gynaecology
University of Turku
Turku
Finland

RESPONSE TO LUTEINIZING HORMONE RELEASING FACTOR (LRF) IN NORMAL SUBJECTS AND ANOVULATORY PATIENTS

Ryosuke Nakano, Fumikazu Kotsuji, Tadayoshi Mizuno, Nobuyuki Hashiba, Motoo Washio and Shimpei Tojo

From the Department of Obstetrics and Gynaecology (Head: Prof. S. Tojo), Kobe University School of Medicine, Kobe, Japan

Abstract. Recently the following decapeptide sequence for porcine luteinizing hormone releasing factor (LRF) was proposed by Schally's group: (Pyro) Glu-His-Trp-Ser-Gly-Leu-Arg-Pro-Gly-NH_2. The polypeptide corresponding to this structure has been synthetized and this synthetic LRF is now available for clinical study. Intravenous administration of synthetic LRF in dose of 200 μg stimulated the rise in serum LH and FSH in normal subjects. Most anovulatory patients showed approximately the same degree of response to LRF. No rise in serum LH or FSH occurred in an anovulatory patient with panhypopituitarism following injection of 200 μg of LRF. It may be concluded that synthetic LRF is convenient means for testing the integrity of the human pituitary for LH and FSH secretion.

The structure of isolated luteinizing hormone releasing factor (LRF) of porcine origin was shown by Schally's group to be (Pyro) Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (1, 4, 12, 13) and the polypeptide corresponding to this structure has been synthetized (5). This synthetic LRF is active in stimulating the secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in rat (12) and in man (2, 7). The polypeptide may represent the hypothalamic releasing factor which controls the secretion of LH and FSH from the anterior pituitary gland.

The synthetic LRF when administered by rapid intravenous or subcutaneous injection, proved to be effective in increasing both LH and FSH levels in subjects in a number of clinical and experimental conditions (2, 3, 14). These observations demonstrated the potential value of LRF in the clinical evaluation of pituitary gonadotrophin secretion. In order to utilize synthetic LRF for this purpose, response to LRF in normal subjects and anovulatory patients was investigated. The purpose of this communication is to report the serum LH and FSH response to LRF in normal subjects and anovulatory patients and to discuss the diagnostic value of LRF for evaluation of pituitary gonadotrophin reserve.

MATERIALS AND METHODS

Before initiating this study toxicity studies with chronic injections of synthetic LRF were performed in rats in order to meet requirements of the Ministry of Health and Welfare.

Clinical subjects

Twenty-one female volunteers were given synthetic LRF. Eight were normal women of reproductive age, four were in the follicular phase and four were in the luteal phase of the menstrual cycle. In addition to normal volunteers, thirteen anovulatory patients showing secondary amenorrhoea were also given synthetic LRF. All were anovulatory patients, the first five showed estrogenic activity and the subsequent eight patients showed no estrogenic activity. According to Matsumoto et al. (6, 7, 8), amenorrhoea due to ovarian insufficiency can be classified clinically into two types; amenorrhoea with estrogenic activity and amenorrhoea without estrogenic activity. The former type has evident estrogen action and uterine withdrawal bleeding occurs after administration of progesterone. The latter type has no estrogen action and uterine bleeding occurs only after combined administration of estrogen and progesterone. This classification has clinical value in the diagnosis and treatment of amenorrhoeic patients.

LRF administration

Synthetic LRF (supplied generously by Daiichi Pharmaceutical Company, Tokyo, Japan) was administered intravenously over 30 sec in a dose of 200 μg to each subject. Blood was sampled at zero time, and at 15, 30,

determination of blood levels and the placental transfer of diazepam in humans. Curr Ther Res 6. 115 1964

4 Ernebo, E., Agurell, S., Jalling, B. & Boreus, L. O. Age differences in drug binding by plasma proteins. Studies on human foetuses, neonates and adults. Eur J Clin Pharmacol *3* 169 1971

5 Flowers, C. E., Rudolph, A. J & Murdina, M M. *Diazepam (Valium) as an adjunct in obstetrical analgesia.* Obstet Gynec *34* 68 1969

6. Friedman, E. A., Niswander K. R. & Sachtleben, M R. Effect of diazepam on labour Obstet Gynec *34* 82, 1969

7 Idänpään-Heikkilä, J E., Jouppila, P I., Puolakka, J O. & Vorne M S. Placental transfer and fetal metabolism of diazepam in early human pregnancy Am J Obstet Gynecol *109* 1011 1971

8. Idänpään-Heikkilä, J E., Taska, R. J Allen, H. A. & Schoolar J G Placental transfer of diazepam- C in mice, hamsters and monkeys. J Pharmacol Exp Ther *176* 752, 1971

9 Marcucci, F Fanelli, R. & Mussini, E. A method for gas chromatographic determination of benzodiazepines. J Chromatogr *37* 318 1968.

10. Moya, F & Smith, B. E. Placental transport of drugs and anesthetics. Anesthesiology *26*. 465 1965.

11 Nisbet, R., Boulas, S. H. & Kantor H L. Diazepam (Valium) during labor Obstet Gynec *29* 726, 1967

12. Page E. W Transfer of materials across the human placenta. Am J Obstet Gynecol *74* 705 1957

13 Schubert, B. & Malmgren, O. Diazepam—en klinisk analytisk undersökning. Opuscula Med *8*. 238, 1969

14 *Timonen, S. & Hagner M. Benzodiazepines in an* unselected obstetrical series. Ann Chir Gynecol Fenn *55* 65 1966.

15 van der Kleijn, E. Kinetics of distribution and metabolism of diazepam in animals and humans. Arch Int Pharmacodyn Ther *182*. 433, 1969

16. Villee, C. A. Placental transfer of drugs. Ann NY Acad Sci *123* 237 1965

Submitted for publication Aug 19 197

Risto Erkkola
Department of Obstetrics and Gynaecology
University of Turku
Turku
Finland

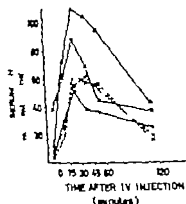


Fig 5 Effect of intravenously administered LRF on serum LH levels in anovulatory amenorrhoeic patients with estrogenic activity

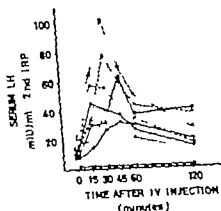


Fig 7 Effect of intravenously administered LRF on serum LH levels in anovulatory amenorrhoeic patients without estrogenic activity

trated in Figs 5 and 6. All patients responded to LRF with a rise of both LH and FSH. The LH peak responses occurred 30 min and the FSH peak responses occurred 45 min after intravenous injection of LRF. The pattern and magnitude of response in these patients were approximately analogous to those of normal subjects (Figs. 5 and 6).

The results in eight anovulatory amenorrhoeic patients without estrogenic activity are shown in Figs 7 and 8. The sole patient with hypopituitarism showed no response to LRF and there was no rise in serum LH and FSH after LRF administration. The other seven patients demonstrated an increase in both LH and FSH. Although the magnitude of the response was not great, there was a clear elevation in both LH and FSH levels (Figs. 7 and 8).

DISCUSSION

The results of the present investigation demonstrate clearly that synthetic LRF given intravenously in man can produce increased LH and FSH levels. The results in four normal follicular phase and four normal luteal phase volunteers showed a serum LH peak 30 min after LRF administration and a FSH peak 45 min after injection. Subsequently LH and FSH levels declined gradually but were still elevated at 2 hours. The present study includes only eight normal subjects and it will be necessary to administer LRF to a considerably larger population before establishing

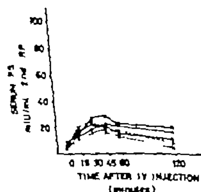


Fig 6 Effect of intravenously administered LRF on serum FSH levels in anovulatory amenorrhoeic patients with estrogenic activity

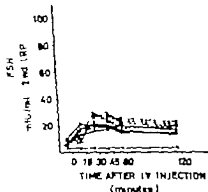


Fig 8 Effect of intravenously administered LRF on serum FSH levels in anovulatory amenorrhoeic patients without estrogenic activity

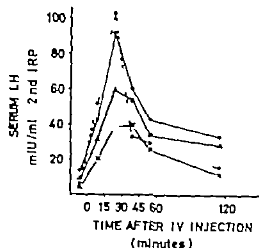


Fig 1 Effect of intravenously administered LRF on serum LH levels in normal subjects in the follicular phase

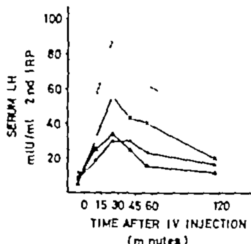


Fig 3 Effect of intravenously administered LRF on serum LH levels in normal subjects in the luteal phase

45, 60, 120 min, for determination of serum LH and FSH concentration. Serum LH and FSH levels were measured in duplicate by double antibody radioimmunoassay according to the methods of Odell et al. (11) and Midgley (9) with modifications. The assay standard was the second international reference preparation of human menopausal gonadotrophin (2nd IRP of HMG) and the results were expressed as mIU/ml.

RESULTS

No serious side effects (change in blood pressure pulse or respiration) were noted in any of the subjects given synthetic LRF

The results in four normal individuals in the follicular phase are shown in Figs. 1 and 2. There was a clear rise of serum LH and FSH in all instances. *The serum LH levels rose from 15 min* and reached a peak 30 min after intravenous administration of LRF The LH levels then declined, but were still elevated _ hours after injection (Fig 1) The serum FSH levels also rose 15 min after LRF administration with a peak after 45 min Subsequently serum FSH levels decreased gradually but were still elevated _ hours after injection (Fig. 2).

The four normal subjects in the luteal phase were also responsive to synthetic LRF and serum LH and FSH levels elevated in all instances (Figs. 3 and 4) They showed approximately the same degree of response to LRF and the pattern seemed to be analogous to that of follicular phase subjects (Figs. 3 and 4) There was no significant difference in the magnitude of response to LRF between follicular and luteal phase subjects.

*The results in the five anovulatory amenor*rhoeic patients with estrogenic activity are illus-

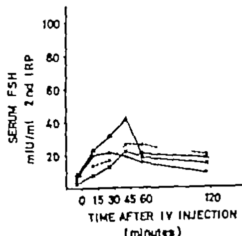


Fig Effect of intravenously administered LRF on serum FSH levels in normal subjects in the follicular phase.

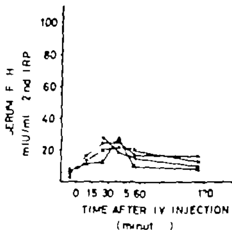


Fig 4 Effect f intra enously administered LRF on serum FSH lev ls in normal subject in the luteal phase

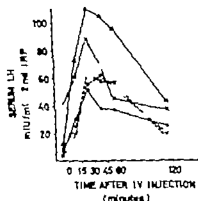


Fig. 5. Effect of intravenously administered LRF on serum LH levels in anovulatory amenorrhoeic patients with estrogenic activity

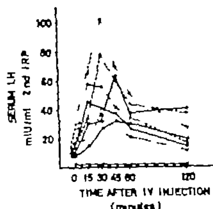


Fig. 7. Effect of intravenously administered LRF on serum LH levels in anovulatory amenorrhoeic patients without estrogenic activity

trated in Figs. 5 and 6. All patients responded to LRF with rise of both LH and FSH. The LH peak responses occurred 30 min and the FSH peak responses occurred 45 min after intravenous injection of LRF. The pattern and magnitude of response in these patients were approximately analogous to those of normal subjects (Figs. 5 and 6).

The results in eight anovulatory amenorrhoeic patients without estrogenic activity are shown in Figs. 7 and 8. The sole patient with hypopituitarism showed no response to LRF and there was no rise in serum LH and FSH after LRF administration. The other seven patients demonstrated an increase in both LH and FSH. Although the magnitude of the response was not great, there was a clear elevation in both LH and FSH levels (Figs. 7 and 8).

DISCUSSION

The results of the present investigation demonstrate clearly that synthetic LRF given intravenously in man can produce increased LH and FSH levels. The results in four normal follicular phase and four normal luteal phase volunteers showed a serum LH peak 30 min after LRF administration and a FSH peak 45 min after injection. Subsequently LH and FSH levels declined gradually but were still elevated at 2 hours. The present study includes only eight normal subjects and it will be necessary to administer LRF to a considerably larger population before establishing

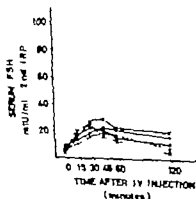


Fig. 6. Effect of intravenously administered LRF on serum FSH levels in anovulatory amenorrhoeic patients with estrogenic activity

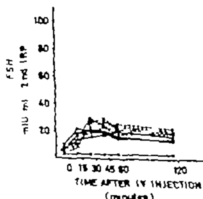


Fig. 8. Effect of intravenously administered LRF on serum FSH levels in anovulatory amenorrhoeic patients without estrogenic activity

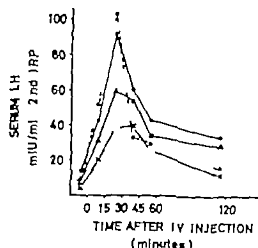


Fig 1 Effect of intravenously administered LRF on serum LH levels in normal subjects in the follicular phase

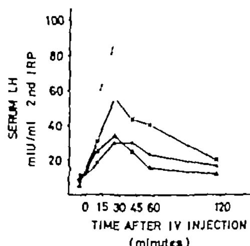


Fig 3 Effect of intra enously administered LRF on serum LH levels in normal subjects in the luteal phase.

45 60, 120 min, for determination of serum LH and FSH concentration. Serum LH and FSH levels were measured in duplicate by double antibody radioimmunoassay according to the methods of Odell et al. (11) and Midgley (9) with modifications. The assay standard was the second international reference preparation of human menopausal gonadotrophin (2nd IRP of HMG) and the results were e pressed as mIU/mL

RESULTS

No serious side effects (change in blood pressure pulse or respiration) were noted in any of the subjects given synthetic LRF

The results in four normal individuals in the follicular phase are shown in Figs. 1 and 2. There was a *clear rise of serum LH and FSH in all in* stances. The serum LH levels rose from 15 min and reached a peak 30 min after intravenous administration of LRF The LH levels then declined but were still elevated 2 hours after injection (Fig. 1) The serum FSH levels also rose 15 min after LRF administration with a peak after 45 min Subsequently serum FSH levels decreased gradually but were still elevated 2 hours after injection (Fig 2)

The *four normal subjects in* the luteal phase were also responsive to synthetic LRF and serum LH and FSH levels elevated in all instances (Figs. 3 and 4) They showed approximately the same degree of response to LRF and the pattern seemed to be analogous to that of follicular phase subjects (Figs. 3 and 4) There was no significant difference in the magnitude of response to LRF between *follicular and luteal phase subjects.*

The results in the five anovulatory amenorrhoeic patients with estrogenic activity are illus-

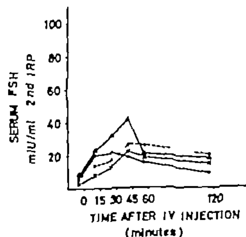


Fig 2 Effect of intravenously administered LRF on serum FSH levels in normal subjects in the follicular phase

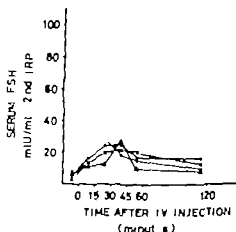


Fig 4 Effect of intra enously dministered LRF on serum FSH levels in normal subjects in the luteal phase

and follicle-stimulating hormones. J Lab Clin Med 70: 971, 1967.

11. Schally A. V, Arimura, A., Kastin, A. J, Matsuo, H., Baba, Y, Redding, T. W, Debeljuk, L., Nair, R. M. G. & White, W. F. A gonadotropin releasing hormone: A single hypothalamic polypeptide hormone regulates the secretion of both LH and FSH. Science *173* 1036, 1971.
12. Schally A. V, Arimura, A., Baba, Y, Nair, R. M. G., Matsuo, H., Redding, T. W & Debeljuk, L. Isolation and properties of the FSH- and LH-releasing hormone. Biochem Biophys Res Commun 43 393, 1971.
13. Schally A. V, Baba, Y, Arimura, A., Redding, T. W & White, W. F. Evidence for peptide nature of LH and FSH-releasing hormones. Biochem Biophys Res Commun 42: 50, 1971.
14. Schally A. V, Kastin, A. J & Arimura, A. Hypothalamic FSH and LH-regulating hormone: structure, physiology and clinical studies. Fertil & Steril 22: 703, 1971.

Submitted for publication Aug. 29, 1972

Ryosuke Nakano
Department of Obstetrics and Gynecology
Kobe University School of Medicine
7 Kusunoki-Cho, Ikuta-Ku
Kobe 650
Japan

with confidence the spectrum of potential responses. However synthetic LRF stimulated both LH and FSH secretion in man and suggests that administration of LRF in a single intravenous injection can provide a convenient test of pituitary gonadotrophin reserve The present results are in agreement with reports of other investigators (2, 3 7)

Five anovulatory amenorrhoeic patients with estrogenic activity responded to administration of LRF with a rise of serum LH and FSH. The pattern and magnitude of serum LH and FSH response were approximately analogous to those of normal subjects. This suggests that pituitary gonadotrophin reserve in amenorrhoeic patients with estrogenic activity is still sufficiently maintained.

Among eight patients with amenorrhoea without estrogenic activity seven showed a response to intravenous injection of LRF and showed the same degree of response The pattern of the serum LH and FSH response was approximately analogous to normal volunteers. Synthetic LRF failed to stimulate serum LH and FSH secretion in the only patient with hypopituitarism. In some anovulatory amenorrhoeic patients without estrogenic activity anovulation is due to pituitary insufficiency and this can be detected by LRF administration In no subject was there any significant toxic effect

We concluded that administration of synthetic LRF in a single intravenous injection of 200 µg provides a test of pituitary gonadotrophin reserve which may prove useful clinically in evaluating and in examining the possibility that there are two types of hypogonadism due to hypothalamo-pituitary disorders: one due to disease of the hypothalamus and one due to disease of the pituitary gland. The results of the present paper suggest that in most cases anovulation is of hypothalamic origin and only few cases of amenorrhoea without estrogenic activity are of pituitary origin

Because of the small numbers of patients, it was not possible to establish a conclusive pattern of responses to LRF in anovulatory patients. However further studies of patients with pituitary lesions should establish more clearly the potential role of LRF in a pituitary function test, and the use of LRF as a diagnostic aid in distinguishing hypothalamic and pituitary lesions may be further refined. Thus, it can be predicted that synthetic LRF will be convenient means for testing the integrity of the human pituitary for LH and FSH secretion.

ACKNOWLEDGEMENT

We wish to thank the National Pituitary Agency and the Endocrinology Study Section of the National Institute of Arthritis and Metabolic Disease for the generous supply of materials for radioimmunoassay of human pituitary gonadotrophins.

The second International reference preparation of HMG was obtained from Dr R. Bangham of the National Institute for Medical Research, London.

REFERENCES

1 Baba, Y Matsuo, H. & Schally A. V Structure of porcine LH and FSH releasing hormone II. Confirmation of the proposed structure by conventional sequential analysis. Biochem Biophys Res Commun 44 459 1971

2 Gual, C., Kastin, A. J Midgley A. R., Jr & Flores, F Administration of LH releasing hormone as a clinical test of pituitary function. The fifty-third meeting of the Endocrine Society (Vol. 88), A 178, 1971

3 Kastin, A. J Schally A. V Gual, C., Midgley A. R., Jr Miller M. C. & Cabera, A.. Dose-response relationship of LH to LH-releasing hormone in man. J Clin Invest 50 1551 1971

4 Matsuo, H Baba, Y Nair R. M G., Arimura, A. & Schally A. V Structure of the porcine LH and FSH-releasing hormone I The proposed aminoacid sequence. Biochem Biophys Res Commun 43 1334, 1971

5 Matsuo, H., Arimura, A., Nair R. M G. & Schally A. V The synthesis of porcine LH and FSH-releasing hormone by the solid phase method. Biochem Biophys Res Commun 45 654 1971

6 Matsumoto, S., Igarashi, M Sato, T Ozawa, M., Inoue S Hosaka, H., Tohma, K., Sato S. & Oka, Y Studies on menstrual disorders. J Jap Obst & Gynec Soc 9 109 196..

7 Matsumoto, S., Igarashi, M Ozawa, M Tohma, K., Sato, T Kubo, H Inoue S Oka, Y & Hosaka, H Studies on menstrual disorders. II. Endocrinological analysis in cases of amenorrhea. J Jap Obst & Gynec Soc 9 161 1962.

8 Matsumoto S Igarashi, M., Sato, T Ozawa, M Ito, T Kribara, M Hosaka, H Sato, S Kubo, H. & Tohma, K.. Studies on menstrual disorders. III. Etiological classification of amenorrhea in 171 cases. J Jap Obst & Gynec Soc 9 40, 1962.

9 Midgley A R., Jr Radioimmunoassay of human follicle stimulating hormone J Clin Endocr 27 295 1967

10 Odell, W D Rayford, P L & Ross, G T Simplified, partially automated method for radioimmunoassay of human thyroid-stimulating, growth, luteinizing

Acta Obstet Gynec Scand 52. 177–181 1973

THE EFFECT OF OXYGEN VENTILATION AND A VASODILATOR ON UTERINE PERFUSION, FOETAL OXYGEN AND ACID-BASE BALANCE

I. *A Study in Healthy Gravidae*

Leo Tervilä, Eero Vartiainen, Inkeri Kivalo, Matti-Johan Pinnilä and Martti Väyrynen

From Departments I and II of Obstetrics and Gynaecology University Central Hospital (Heads: Prof S. Timonen and Prof P. Vara), Helsinki, Finland

Abstract. Uterine perfusion, the p_{O_2} in maternal ear capillary blood, the p_{O_2} and pH in portio blood, and the p_{O_2}, pH, BE and p_{CO_2} in blood from the foetal scalp were investigated in 50 normal women in the 36th or subsequent weeks of pregnancy. Women were given either oxygen ventilation, xanthinol nicotinate (Complamin®), or oxygen ventilation in conjunction with this drug. Uterine perfusion decreased in all groups, the greatest fall being in the group ventilated with 100% oxygen. The p_{O_2} rose in all groups given oxygen. The smallest rise was noted in foetal blood. The remaining changes in blood chemistry were very small.

It has been demonstrated both in animals and in human beings that a rise of the p_{O_2} in maternal blood is accompanied by a rise of the foetal p_{O_2} (1 2, 11 12, 14 16). However the elevation of the latter is relatively slight, the mean being 10–15 mmHg (8). Moreover when the maternal p_{O_2} rises above 200 mmHg, the concentration in the foetal blood no longer follows the maternal concentration (7). This phenomenon has been attributed to an impairment of the utero-placental circulation due to vasoconstriction. Such a sequence of events has been reported in animal experiments (8, 15) and in human isolated blood vessels and isolated placentas studied *in vitro* (13). On the other hand an impaired acid-base balance has been observed e.g. in association with hyperventilation and positive pressure ventilation. Hence caution seems to be indicated with regard to oxygen exhalation of the mother in the treatment of foetal asphyxia (9 10 16). It has proved possible in part to eliminate the above-mentioned untoward effect by the administration of a vasodilator in conjunction with oxygen (16 18).

In this study the changes in uterine perfusion and foetal acid-base balance were measured *in vivo* in gravidae given oxygen and a vasodilator. An attempt was made to eliminate the possible effect of factors not taken into account in previous investigations. The patients were therefore chosen from normal gravidae in the last four weeks of pregnancy.

MATERIAL AND METHODS

The series consisted of 50 normal gravidae with gestation period of at least 36 weeks, admitted to Departments I and II of Obstetrics and Gynaecology University Central Hospital, Helsinki, who were not in labour but either had ruptured membranes or were being subjected to amniotomy. All abnormal cases were rejected, including patients with haemoglobin concentration below 11 g/100 ml or blood pressure above 140/90 mmHg. Moreover, cases in which the baby was given a one-minute Apgar score under 7 and cases with an initial foetal pH value of 7.25 or lower in micro blood samples were omitted.

The patients were divided into groups as follows. 1) 7 patients, serving as controls, were ventilated with air by mask with an anaesthesia machine. 2) 14 patients were ventilated with 100% oxygen, 3) 13 patients were given 300 mg. xanthinol nicotinate (Complamin®)* intramuscularly. 4) 3 patients were given Complamin intramuscularly and 100% oxygen 3–5 min later. In addition, 3 patients were given 50% oxygen-nitrogen mixture, 3 patients were given mixture of oxygen and 4% CO_2, and 2 patients received 8% CO_2. All the gases were given by mask with an anaesthesia machine using semiclosed system with CO_2 absorber.

* Xantinol nicotinate (Complamin®) used in this study was kindly supplied by Johann A. Wülfing, Düsseldorf, Western-Germany.

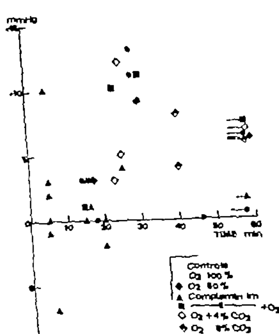


Fig. 3. Changes in p_H in capillary blood from the portio. The averages on the right.

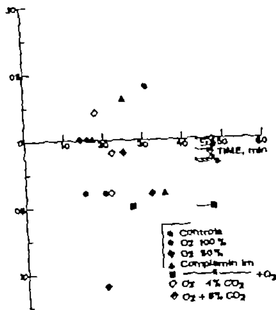


Fig. 4. Changes in p_{O_2} in capillary blood from the foetal scalp. The averages on the right.

greater in the 100 % oxygen group than in the Complamin® group ($p < 0.001$). The difference between the combined group and the Complamin® group was significant ($p < 0.01$).

With regard to the p_H in portio blood the differences between the groups were very small (Fig. 4). In foetal blood the range was somewhat wider (Fig. 5). A statistically significant difference was only noted between the groups given a CO_2 mixture and the groups given oxygen or Complamin®. No significant difference was obtained between the oxygen and Complamin® groups.

The BE in foetal blood was of the same level in all groups (Fig. 6). The greatest variations were observed in the p_{CO_2} groups (Fig. 7). A statistically significant difference was obtained between the combined and Complamin® group, an increase in p_{CO_2} being noted in the former a decrease in the latter. In the oxygen-ventilated groups no changes occurred during the observation time.

The curves representing the changes in uterine perfusion are given in Fig. 8. No statistically significant differences between the groups were obtained, but the curves show that tissue perfusion deteriorated in all groups during the observation time. The slightest fall was observed in the control group in which perfusion rapidly returned to the initial level. Circulation deteriorated in the groups given oxygen, the changes occurring very rapidly after the initiation of oxygen ventilation.

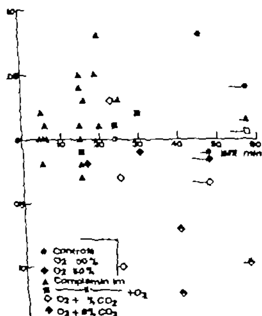


Fig. 5. Changes in p_H in capillary blood from the foetal scalp. The averages on the right.

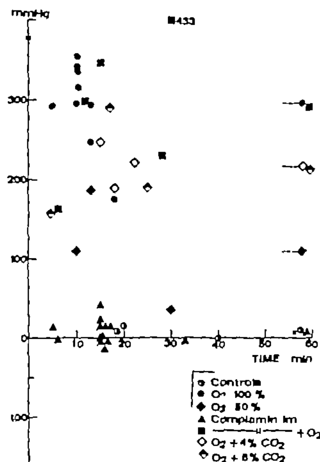


Fig. 1. Changes in p_{O_2} in capillary blood from the ear of the mother. The averages on the right.

Before and at different intervals during treatment micro-blood samples were drawn from the ear, the portio and the foetal scalp by Saling's technique without preliminary hyperaemia. In order to avoid stress to the patients only initiated and final samples were usually collected, instead of serial samples. The samples from the ear were used for the determination of p_{O_2} and the samples from portio p_{O_2} and p_H, p_{CO_2} and BE were determined in foetal blood. Duplicate samples were used throughout. The glass capillary was placed on ice and investigations were performed immediately after sample taking. The p_{O_2} was determined electrometrically with an oxygen electrode. The remaining investigations were performed by the Astrup method.

Uterine perfusion was measured with a thermistor needle inserted into the portio, at intervals of a few minutes according to the principle of thermodilution (5). The original technique of Brotanek was modified, only one needle being used. A needle about 0.5 mm in diameter with a tip furnished with a thermistor commercially manufactured for the measurement of tissue temperatures (Yellow Springs Instrument Company Inc., Ohio, USA), was used.

The tissue temperature was first measured using very low voltage by which the thermistor needle was practically unheated. Then, measurement was repeated using a higher voltage which warmed the needle-tip about 1°C. The same voltages were used throughout. Under these circumstances the change in temperature of the needle-tip may be considered a measure of the thermodiluting capacity of the tissue, which is inversely proportional to tissue perfusion.

It has proved difficult to determine the level of tissue perfusion by this method, while changes in perfusion are readily recorded. As regards the level, the results are influenced by the distribution of large blood vessels and other factors not directly related to perfusion. These may cause relatively wide fluctuations in the thermodiluting capacity. However, when the changes in tissue perfusion are followed for a short time and all measurements are performed by one insertion of the needle, this is of little consequence.

In each experimental series the changes in perfusion after 2, 5, 8, 12, 17 and 23 min were calculated by linear interpolation. Extrapolation was not made, and the number of observations therefore decreased with the lapse of time. Mean values for all groups were calculated.

RESULTS

The p_{O_2} values in the various maternal and foetal samples were higher in the groups given oxygen than in the Complamin® and control groups (Figs. 1, 2, 4). Owing to the scanty material the differences in the auricular samples only attained the level of almost significant, using the *t*-test. The foetal value was highly significantly

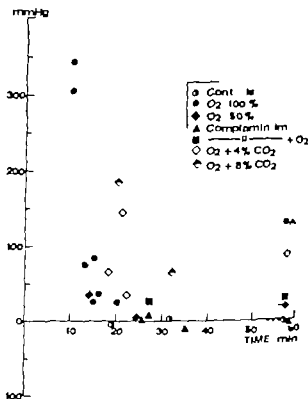


Fig. 2. Changes in p_{O_2} in capillary blood from the portio. The averages on the right.

ventilation and thus eliminated the retention of acid metabolic products. The present results argue in favour of the view that Complamin® given in conjunction with oxygen has practically no dilating effect on the portio capillaries in normal gravidae.

Although Complamin® in conjunction with oxygen caused a rise of the p_{O_2} in foetal blood, no change in BE was observed in these cases. Obviously the observation time was too short for signs of metabolic acidosis to develop.

According to Schmidt (17), a fall of the O_2 level and a rise in CO_2 in the blood are essential characteristics of asphyxia. If this definition is abandoned in favour of the modern view that the degree of asphyxia depends on the p_H and the p_{CO_2} and BE levels in the blood (3, 6 7), the conclusion may be drawn that oxygen ventilation has no appreciable effect. However it should be borne in mind, that the present series consisted of gravidae in good condition with normal initial blood chemistry. Additional information with regard to the effect of oxygen ventilation is only obtained by investigating a series of initially asphyxiated babies.

REFERENCES

1 Althabe, O Schwarcz, R. L., Pose, S. V. Escarcena, L. & Caldeyro-Barcia, R.. Effects on fetal heart rate and fetal p_{O_2} of oxygen administration to the mother Am J Obstet Gynec 98 858, 1967

2. Baraka, A. Correlation between maternal and foetal p_{O_2} and p_{CO_2} during caesarean section. Brit J Anaesth 42 434, 1970

3 Beard, R W Morris, E. D. & Clayton, S. G pH of foetal capillary blood as an indicator of the condition of the foetus J Obstet Gynaec Brit Comm 74 812, 1967

4 Bernacher, J & Saling, E. Der Einfluss einer Sauerstoffatmung der Mutter auf den Feten. Anaesthesist 15 136, 1966

Bencinek, V Kanda, S & Roth, L.. A method for studying uterine blood flow in pregnant women. Physiol Bohemoslov 11 358, 1962

6 Hon, E. H Khazin, A. F & Paul, R. H Biochemical studies of the fetus, II Fetal pH and Apgar scores Obstet Gynec 33 237 1969

7 James, L. S Weisbrot, I. M., Prince, C. E., Holaday D. A. & Apgar, V The acid-base status of human infant in relation to birth asphyxia and the onset of respiration. J Pediatr 52 379 1958.

8. Kirschbaum, T. H Lucas, W E., DeHaven, J C. & Assali, N. S.. The dynamics of placental oxygen transfer. I. Effects of maternal hyperoxia in pregnant ewes and fetal lambs Am J Obstet Gynec 98 429 1967

9 Morishima, H. O Daniel, S. S Adamsons, K. & James, L. S.. Effects of positive pressure ventilation of the mother upon the acid-base state of the fetus. Am J Obstet Gynec 93 269 1965

10 Motoyama, E. K., Rivard, G Acheson, F & Cook, C. D Adverse effect of maternal hyperventilation on the fetus Lancet 1 286, 1966.

11 Motoyama, E. K., Rivard, G., Acheson, F & Cook, C. D The effect of changes in maternal pH and p_{CO_2} on the p_{O_2} of fetal lambs. Anesthesiology 28. 891 1967

12. Newman, W McKinnon, L., Philips, L., Paterson, P & Wood, C.. Oxygen transfer from mother to fetus during labor Am J Obstet Gynec 99 61, 1967

13 Nyberg, R. & Westin, B The influence of oxygen tension and some drugs on human placental vessels. Acta Physiol Scand 39 216, 1957

14. Parker H, R. & Purves, M J Some effects of maternal hyperoxia and hypoxia on the blood gas tensions and vascular pressures in the foetal sheep. Q J Exp Physiol 52 205 1967

15 Rivard, G Motoyama, E. K Acheson, F M., Cook, C. D & Reynolds, E. O R.. The relation between maternal and fetal oxygen tensions in sheep. Am J Obstet Gynec 97 925, 1967

16 Saling, E. Die Wirkung einer O_2-Atmung der Mutter auf die Blutgase und den Säure-Basen-Haushalt des Feten Geburtsh Frauenheilk 23 528, 1963.

17 Schmidt, C. F Medical physiology (ed Philip Bard). Mosby St. Louis, 1956.

18. Tervila, L. R., Vartiainen, E., Pasila, M. J O., Kivalo, M. I & Väyrynen, M. A The effect of variations in the oxygen content of maternal blood on the perfusion of the uterus and on the oxygen and Astrupp values of the fetus Acta Obstet Gynec Scand 50 Suppl 9 73 1971.

Submitted for publication Sept 24 1972

Leo Tervila
Department of Obstetrics and Gynaecology
University Central Hospital
Helsinki
Finland

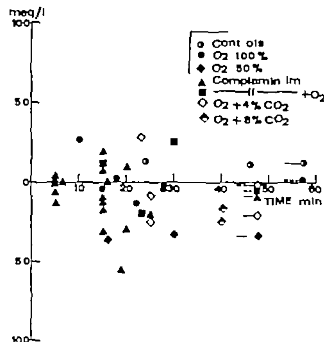


Fig 6 Changes in BE in capillary blood from the foetal scalp. The averages on the right.

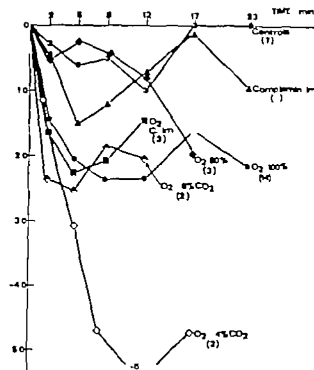


Fig 8 Changes in uterine perfusion. Number of cases in brackets.

DISCUSSION

The p_{O_2} rose in blood from all sites investigated when the mother was oxygen ventilated. However the rise was clearly lower in portio blood than in auricular samples, and lower still in foetal blood. This finding, which has previously been reported, seems to be related to impaired uterine perfusion. The results were not appreciably influenced by the administration of vasodilator in conjunction with oxygen. When the oxygen concentration in the ventilation gas was 50 % the rise in p_{O_2} in auricular and portio blood was lower than in the mothers given 100 % oxygen. However p_{O_2} in foetal blood was of the same level in both groups. Complamin alone did not influence p_{O_2}.

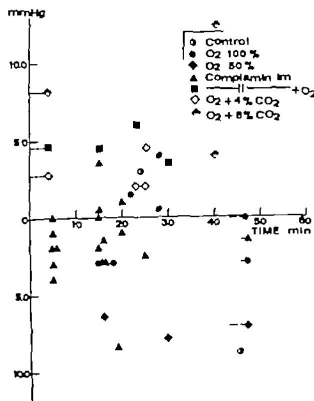


Fig 7 Changes in p_{CO_2} in capillary blood from the foetal scalp. The averages on the right.

The p_H in maternal blood did not seem to vary in any of the test groups, and the administration of oxygen and Complamin® in conjunction caused no appreciable change in the foetal acid-base balance. By contrast when the ventilation gas contained CO_2 the p_H values fell considerably.

Although oxygen ventilation had no appreciable effect on p_{CO_2} in foetal blood, a rise was observed when Complamin was given in conjunction with oxygen. The value then obtained was also clearly higher than in the group given Complamin® alone. This manifestation of acidosis is not readily explained. In previous investigations (4, 16) the administration of vasodilators counteracted the vasoconstriction of the uterus caused by oxygen

Acta Obstet Gynec Scand 52: 183–185 1973

TOXAEMIA AND CIGARETTE SMOKING DURING PREGNANCY

Prospective Consecutive Investigation of 3 927 Pregnancies

Bertil Palmgren, Tore Wahlén and Bo Wallander

From the Department of Obstetrics and Gynaecology (Head: Docent T. Wahlén), Helsingborg Hospital, Helsingborg, Sweden

Abstract. Toxaemia of pregnancy occurs in 1 out of every 10 pregnant non-smokers, compared with 1 out of every 20 smokers. This might be due to the abortifacient effect of smoking, especially in the first trimester with consequent exclusion of preeruptive candidates for toxaemia. Another interesting possibility is that the cyanides included in tobacco smoke are converted to thiocyanates and, theoretically might offer certain protection against thrombosis in the placenta and against toxaemia. The perinatal mortality among low-weight newborns (2 500 g) is much higher among infants of non-smokers, as is the frequency of bleeding and infarction of the placenta. This suggests that the perinatal mortality among low-weight newborns of mothers who are non-smokers is to larger extent due to toxaemia than is that among infants of smokers.

In recent years considerable interest has been focused on the effect of cigarette smoking during pregnancy on the prognosis of the foetus. All investigations have shown that such smoking results in lower birthweight of the child (about 200 g) and a higher frequency of premature births. In heavy smokers also the frequency of abortion is increased. Other simultaneous factors, such as poor social environment, conflict situations, increased use of various sorts of pills etc. may also influence the prognosis of the foetus. It was therefore considered of interest to assess the effect of smoking and other factors capable of influencing pregnancy even though it is, of course, not possible to study each factor separately.

The correlations between toxaemia of pregnancy low birthweight and foetal death are well know.

One might imagine that cigarette smoking increases the frequency of toxaemia by the vasoconstrictive effect of cigarette smoke. Underwood et al. (7) tested this hypothesis in a prospective investigation and arrived at the surprising conclusion that the frequency of toxaemia was not increased, but decreased, in pregnant women who smoked. This was confirmed by Duffus & Gillivray (3) in a similar prospective investigation of 2543 pregnancies. On the other hand, low birthweight and perinatal mortality were noted less often among children of toxaemic women who were smokers than among those who were not.

In a prospective study of 6363 pregnancies Kullander & Källén (5) found preeclampsia to be less common among smokers (11 %) than among non-smokers (16 %).

Since the number of published investigations is small and since the phenomenon cannot be regarded as sufficiently elucidated, we thought it justified to try further to elucidate this possible "protective effect of cigarette smoking on toxaemia of pregnancy. The material is the same as that of the prospective perinatal investigation carried out at the Department of Gynaecology and Obstetrics and the Department of Paediatrics in Helsingborg and reported by Enell & Wahlén in Svensk Läkartidning, 1971 (4)

MATERIAL

The investigation, which covered 3-year period (1964–1967), was carried out on all women whose pregnancies were diagnosed by the gynaecologists at the Department of Obstetrics and by the practising gynaecologists in the town and who were delivered at the Department of Obstetrics, Helsingborg. All of the infants born were examined by physicians at the Department of Paediatrics.

Acta Obstet Gynec Scand 52, 183–185 1973

TOXAEMIA AND CIGARETTE SMOKING DURING PREGNANCY

Prospective Consecutive Investigation of 3 927 Pregnancies

Bertil Palmgren, Tore Wahlén and Bo Wallander

From the Department of Obstetrics and Gynaecology (Head, Docent T. Wahlén), Helsingborg Hospital, Helsingborg, Sweden

Abstract. Toxaemia of pregnancy occurs in 1 out of every 10 pregnant non-smokers, compared with 1 out of every 20 smokers. This might be due to the abortifacient effect of smoking, especially in the first trimester, with consequent exclusion of presumptive candidates for toxaemia. Another interesting possibility is that the cyanides included in tobacco smoke are converted to thiocyanates and, theoretically, might offer certain protection against thrombosis in the placenta and against toxaemia. The perinatal mortality among low-weight newborns (2 500 g) much higher among infants of non-smokers, as is the frequency of bleeding and infarction of the placenta. This suggests that the perinatal mortality among low-weight newborns of mothers ho are non-smokers is to larger extent due to toxaemia than is that among infants of smokers.

In recent years considerable interest has been focused on the effect of cigarette smoking during pregnancy on the prognosis of the foetus. All investigations have shown that such smoking results in lower birthweight of the child (about 200 g) and higher frequency of premature births. I heavy smokers also the frequency of abortion is increased. Other simultaneous factors, such as poor social environment, conflict situations, increased use of various sorts of pills etc., may also influence the prognosis of the foetus. It was therefore considered of interest to assess the effect of smoking and other factors capable of influencing pregnancy even though it is, of course, not possible to study each factor separately.

The correlations bet een toxaemia of pregnancy low birthweight and foetal death are well know.

One might imagine that cigarette smoking increases the frequency of toxaemia by the vasoconstrictive effect of cigarette smoke. Underwood et al. (7) tested this hypothesis in a prospective investigation and arrived at the surprising conclusion that the f equency of toxaemia was not increased, but decreased, in pregnant women who smoked. This was confirmed by Duffus & Gillivray (3) in a similar prospective investigation of 2543 pregnancies. On the other hand, low birthweight and perinatal mortality were noted less often among children of toxaemic women who were smokers than among those who were not.

In a prospective study of 6363 pregnancies Kullander & Källén (5) found preeclampsia to be less common among smokers (11%) than among non-smokers (16%).

Since the number of published investigations is small and since the phenomenon cannot be re garded as sufficiently elucidated, we thought it justified to try further to elucidate this possible "protective effect of cigarette smoking on tox aemia of pregnancy. The material is the same as that of the prospective perinatal investigation car ried out at the Department of Gynaecology and Obstetrics and the Department of Paediatrics in Helsingborg and reported by Enell & Wahlén in Svensk Läkartidning, 1971 (4).

MATERIAL

The investigation, which covered 3-year period (1964–1967), was carried out on all women hose pregnancies were diagnosed by the gynaecologists at the Department of Obstetrics and by the practising gynaecologists in the town and who were delivered at the Department of Obstetrics, Helsingborg. All of the infants born were ex amined by physicians at the Department of Paediatrics.

Table I *Frequency of prematurity (birthweight <2 500 g) in 3 927 infants of smokers and non-smokers*

	Non-smokers	<10 cig.	>10 cig.	Total
Total no. of children	2 087	1 486	354	3 927
Children <2 500 g	68 3.3 %	97 6.5	22 6.2	187 4 8

RESULTS

Table I shows the frequency of prematurity which was clearly higher for the smokers, 6.5 and 6.2 % than for the non-smokers, 3.3 % These figures agree well with those found in series studied by other investigators. The mean birth weight of the infants of smokers was 220 g lower than that of children of non-smokers.

The perinatal mortality of the premature infants (<2 500 g = the internationally accepted criterion for prematurity) is given in Table II. The mortality was higher 29 5 % among children of non-smokers than of smokers, 16.5 and 18 2 % respectively

The table also shows that bleeding and infarction of the placenta was definitely more common among the non-smokers: 23.5 and 16.2 % respectively than among smokers, 14 4 and 11 3 % for the light smokers (<10/day) 18.2 and 4 6 % for the heavier smokers. The figures are of interest because experience shows that bleeding and infarctions in the placenta occur more often in women with toxaemia.

Table II *Perinatal mortality (stillborns and infants who died within 7 days) and placental complications of 187 premature deliveries (infants below 2 500 g) distributed among smokers and non-smokers*

	Non-smokers	<10 cig	>10 cig.
No. of children below 2 500 g	68	97	22
Perinatal mortality	20 29.5	16 16.5 %	4 18 2
Bleeding in placenta	16 23.5 %	14 14 4 %	4 18.2
Infarcts in placenta	11 16.2 %	11 11.3 %	1 4 6

Table III *Incidence of symptoms of toxaemia and oedema during 3 927 pregnancies terminating in birth of child*

	Non-smokers	<10 cig.	>10 cig
Albuminuria, hypertension, preeclampsia, eclampsia	219 10.5 %	123 8.3	1 5.9
Oedema	475 22.8 %	309 20 8 %	79 ... 3

It might be mentioned that in the investigation by (5) for smokers the mean placental weight decreased less than the mean birthweight of the children compared with the corresponding values for the non-smokers.

The frequency of toxaemia (albuminuria hypertension preeclampsia, eclampsia) in the groups of smokers and non smokers in the material is given in Table III. The figures obtained are practically the same as those reported by (3 6) i.e. a higher frequency of toxaemia among non-smokers (10.5 %) than among moderately heavy smokers (8 3 %) and among heavy smokers (5.9 %). The differences were statistically significant.

On the other hand, no difference was found between the frequency of oedema as the only possible symptom of toxaemia. Treatment of toxaemia usually includes administration of diuretics and as is clear from Table IV the consumption of diuretics was greater among non-smokers (38 4 %) i.e. the consumption tended to be lower among smokers than among non-smokers.

DISCUSSION

It is difficult to explain why toxaemia of pregnancy was less common among smokers (1 out of 20) than among non-smokers (1 out of 10). Two of the research teams, who had made the same observation as we suggested the existence of

Table IV *Consumption of diuretics in third trimester of 3 927 pregnancies terminating in birth of child*

	Non-smokers	<10 cig	10 cig.
Diuretics	801 38 4	507 34.2	119 33 6

Table V *Pregnancies lost among smokers and non-smokers*

	Non-smokers	<10 cig	>10 cig	Total
Total	2 264	1 634	414	4 312
Early abortion	157	131	54	342
	6.9	8.0%	13.0	9%
Late abortion	20	17	6	43
	0.9%	1.1%	1.5	1.0%
Prenatal mortality	17	7	5	29
	0.8%	0.5%	1.4%	0.7%
Neonatal mortality	11	13	2	26
	0.5%	0.9%	0.6%	0.6%
Pregnancies lost	205	168	67	440
Total	9.6%	10.3%	16.2%	10.2%

some protective mechanism against toxaemia in smokers, but they said nothing about the possible nature of such a mechanism. One might imagine the occurrence of some form of interaction between components of the smoke and enzymatic processes in the metabolism. In this connection it should be recalled that cyanide is an active component of tobacco smoke and that thiocyanate is the main detoxication product in vivo (2). According to J. Wilson (8) and others, thiocyanate has hypotensive effect and stimulates renal function (increased clearance values), which might imply a favourable effect on mothers with hypertension and albuminuria.

Another possible explanation might be an abortive effect of smoking, especially in the first trimester with the result that the number of presumptive candidates of toxaemia lost might be larger among the smokers than among the non-smokers. Table V shows that in the women who smoked more than 10 cigarettes day the frequency of abortion was twice as high as among non-smokers. The frequency of abortion plus that of toxaemia was the same among smokers as among non-smokers, when corrected for the number of children in the respective group: 208 abortions + 144 cases of toxaemia among 2 048 smokers, compared with 177 abortions + 219 toxaemia among 2 264 non-smokers. The preponderance of abortions among the smokers might thus also explain the difference.

The finding of a higher frequency of toxaemia among non-smokers is in accord with a larger consumption of diuretics in this group.

The higher perinatal mortality among children of non-smokers and weighing less than 2 500 is also in accord with the increased occurrence of bleeding and infarctions in the placenta in non-smokers. In this context it is interesting to note that Astrup & Stage (1) showed that a fibrinolytic activator can be extracted from tissues with the aid of potassium thiocyanate. An increased amount of such activator substance in the blood may possibly mean an increased protection against thrombosis in the placenta and against hyaline membranes in the bronchi of the infants. In addition, these infants are probably really premature while some of the low weight infants of mothers who were smokers were small for dates rather than premature.

REFERENCES

1. Astrup, T. & Stage, A., Nature *170*: 929, 1952.
2. Boxer, G. E. & Richards, J. C.: Archs Biochem *39*: 7, 1952.
3. Duffus, G. M. & MacGillivray, I.: Lancet *1*: 994, 1968.
4. Enell, H. & Wahlén, T.: Sv Läkartidn *22*: 2605, 1971.
5. Kullander, S. & Källén, B.: Acta Obstet Gynec Scand *50*: 83, 1971.
6. Palmgren, B. & Wallander, B.: Sv Läkartidn *22*: 2611, 1971.
7. Underwood, P. B. et al.: Obstet Gynec *29*: 1, 1967.
8. Wilson, J.: Lancet *2*: 1157, 1968.

Submitted for publication Sept. 26, 1972

Bo Wallander
Stortorget 13
252 20 Helsingborg
Sweden

Acta Obstet Gynec Scand 52: 187–189 1973

CASE REPORT

UTERUS DIDELPHYS WITH AN ABORTION INTO A UNILATERAL IMPERFORATE VAGINA

Karl-Gösta Nygren and Bengt H. Persson

From the Department of Obstetrics and Gynecology (Head: Prof. Carl Gemzell), University Hospital, Uppsala, Sweden

Abstract. A case of abortion into unilateral imperforate vagina of uterus didelphys is reported. This abnormality is very rare and a pregnancy in the uterine horn on the imperforate side seems not to have been reported before. No communication into the "hidden" horn of the uterus except the transperitoneal route was found. Therefore, conception must have occurred by transperitoneal migration either of spermatozoa or of fertilized egg.

This case is of interest not so much for the presence of a genital malformation as for the mechanism of conception and the confusion of diagnosis resulting from the "hidden abortion within the imperforate vagina.

CASE REPORT

C. R., aged twenty-four. Menarche at the age of 13. Her menstruations were always rather heavy with moderate pains mostly located to the left side in the lower abdomen. Except for this discomfort she had always been healthy. In 1967 she saw doctor because of moderate vaginal discharge. A previously unknown cystic mass was detected to the left of small uterus. The cyst reached down into the left paracolpos and bulged into the upper part of the vagina. She was admitted to gynaecological clinic where pelvic examination revealed soft cystic, hen egg-sized mass which bulged down in the left upper part of the narrow vagina. A normal uterus was palpated to the right of the cyst.

Laparotomy was performed and double uterus with horns of equal size was explored. Far down in the left parametrium and paracolpos the soft cystic mass could again be recognized but its real nature could not be revealed. The cyst could be partly compressed and fistula to the bladder was then suspected. No connection with the vagina could be revealed. No further attempts to explore the cyst by surgical intervention were made.

After the laparotomy the following examinations were performed:

1. Cystoscopy—no diverticulum or fistula could be seen; no ureteric orifice was visible on the left side.

2. Intravenous urography revealed normal kidney on the right side but no signs of kidney or ureter on the left side.

3. Hysterosalpingography—visualized well developed right horn of the uterus with patent tube, with the opaque medium disseminating freely in the peritoneal cavity. No connection with the left horn of the uterus could be seen (Fig. 1).

In April 1970 the patient was admitted to the gynaecological department of the University Hospital of Uppsala because of vaginal bleeding and low back pain. She was then pregnant in the 13th week. On pelvic examination slight, dark bleeding could be seen from closed cervix, which was displaced to the right by bulging soft mass in the upper left fornix of the vagina (Fig. 2). On palpation the uterus was firm, slightly enlarged and displaced to the right by soft, diffuse, tender mass on the left of the pelvis. This mass bulged down into the cystic vaginal tumour seen on vaginal examination. HCG in the urine was found to be 60 000 IU/l.

The clinical picture was confusing but an imperforate vagina on the left side was suspected. A vaginal operation was performed and an incision was made over the bulging area in the left wall of the vagina. The cystic mass was then found to be an imperforate vagina filled with blood and completely expelled conceptus. Slow continuous bleeding came from the cervix opening into the left vagina. No signs of infection were found. Curettage of both uterine cavities was performed and the separating wall between the two vaginae was removed. Macroscopical as well as histological examination of the specimen from the left horn of the uterus revealed typical abortion with small foetus. The patient left the hospital week later after an uneventful postoperative period. Four months later she was pregnant again in the same left horn of the uterus. No complications occurred during this pregnancy. A boy of 2 910 g was born by vertex presentation three weeks before the expected time of delivery.

[illegible] some histomorphological aspects. Studentlitt., Lund, [illegible]

2. Allen, K. & Cowan, L. E. Uterus didelphys with unilateral imperforate vagina. Obst Gynecol 22, 422, 1963

3. Gordon, C. A. Ruptured pregnancy in the closed rudimentary horn of a bicornate uterus. Amer J Obstet Gynec 29 273, 1935.

4. [illegible], L. W. Uterus duplex. Amer J Obstet Gynec 25, [illegible] 1933.

5. [illegible], K. Uterus didelphys with unilateral haematocolpos. J Obst Gynaec Brit Comm 79 85 1972.

6. Melander, S. Malformations of uterus and vagina. Nord Med 63 506, 1960.

Submitted for publication June 15 1972

Karl-Gösta Nygren
Kvinnokliniken
Akademiska sjukhuset
S-750 14 Uppsala 14
Sweden



Fig 1 Hysterosalpingography of the right horn of the uterus before the imperforate vagina was opened. No communication to the left side could be demonstrated.

DISCUSSION

Uterus didelphys with a unilateral imperforate vagina is a very rare abnormality (2 5) In a recent paper Johansen (5) could find no previous reports in the literature of the condition associated with pregnancy but described one case with a pregnancy in the horn of the uterus connected with the open vagina. Pregnancy on the "imperforate side of this abnormality has to our knowledge not been reported before. The left uterine horn was fully capable of providing accomodation for a full-grown fetus as this was accomplished a short time after removal of the vaginal septum.



Fig 2 The appearance at vaginal examination t the time of admittance to the University Hospital. The cervix of the nonpregnant right horn of the uterus was pushed to the right by the bulging cystic mass in the left paracolpos.

As in all other cases of this rare condition (5) the kidney on the imperforate side was aplastic or hypoplastic.

The absence of symptoms of the condition before pregnancy occurred is typical for many abnormalities of the genital tract (6).

According to Gordon (3) pregnancies in closed rudimentary horns of bicornuate uteri must have occurred by transperitoneal migration of spermatozoa or by entrance of a fertilized egg from the contralateral ovary into the tube of the rudimentary horn As we did not recognize the site of the corpus luteum in the present case both these possibilities must be left open. The occurrence of spermatozoa in the abdominal cavity of women has been reported by Ahlgren (1) and others.

REFERENCES

1 Ahlgren, M M gration f spermatozoa to the Fallopian tubes and the abdominal cavity in women in-

NEW INSTRUMENTS

THE ANKER DILATOR IN THERAPEUTIC ABORTION[1]

Bo von Friesen

From the Department of Obstetrics and Gynecology (Head. Bo von Friesen), Central Hospital, Lidköping, Sweden

In 1958, Herman Anker (1) described a new type of cervical dilator which was intended to supersede the instrument introduced by Lövset during the nineteen-thirties, as well as—to some extent—the Hegar dilator. The new dilator was simpler and no less effective: it had been used by Anker as a prelude to curettage in more than 800 cases.

The instrument is essentially hairpin-shaped spring (Fig. 1). The two limbs, hich are inserted into the uterus, are cm long, transversely serrated on the outside and flat on the inside. When in apposition, the limbs ar together equivalent in size to no. 4 Hegar dilator. The proximal part of the instrument which lies in the vagina, angled at 30° to the limbs; it is 9 cm long and is made of round steel of 3.5 mm diameter. The limbs are held in apposition by retaining clip. hen this removed the limbs spring apart by up to 3 cm and can exert pressure somewhat in excess of 1 kg.

W have used the Anker dilator as a prelude to the evacuation of early abortions since 1960. Before the hypertonic saline method came into general use, w also used the instrument as a prelude to partial evacuation. The instrument is normally inserted into the cervical canal on the afternoon of the preoperative day. When the retaining clip is removed, the patient feels a sensation of pressure but ery seldom of slight pain. It is, in fact, remarkable how extremely little discomfort is experienced.

In 83% of 115 parous women, the patency achieved was sufficient to admit Saenger abortion forceps or a no. 14 (the largest) vacuum suction catheter, regardless of whether or not the pregnancy had progressed beyond the 12th week. In 46 nulliparous women the corresponding figure was 72%.

A vaginal pack was normally sufficient to prevent the instrument slipping out of place—which was one cause of failures. In 16 patients, this method permitted us to perform primary evacuation from the 13th up to the 16th week. There were no immediate complications attributable to the Anker dilator.

Adequate dilatation permits easy and saf curettage since big instruments can be used. It is vital, however, to know to what extent cervical damage may be inflicted by such dilatation.

In the literature, the author has been able to find only a few references to the incidence of cervical tears with Hegar dilators; two reports were from Germany: Lork (4) and Helbing (2) reported incidences of 2.4% and 4.2% respectively in connection with therapeutic abortion. No further details were available. An extensive study by Hulka & Higgins (3) revealed that the use of Hegar dilators prior to curettage frequently resulted in cervical lacerations; 154 such patients subsequently underwent hysterectomy and the cervices were carefully examined. The degree of dilatation performed was unfortunately not recorded. In 39 ± 6.8% the internal os was torn to depth of more than 2 mm and in 22 ± 6.8% to a depth of more than 5 mm. These tears were all of characteristic appearance

---

[1] In personal communication, Professor A. Majewski of Hannover has disclosed that similar dilator was introduced in Germany as long ago as the turn of the century. Majewski still uses this instrument, which he considers very useful in certain cases of abortion.

The cervix was regularly dilated to Hegar 10–12 (J. F. Hulka, pers. com.)

ANNOUNCEMENTS

GERMAN SOCIETY FOR ENDOCRINOLOGY COMPETITIONS FOR 1974

Awards

Schoeller Junkmann Award

Amount DM 15 000 —

Donator Schering AG Berlin

Applicants must reside in Europe and be not older than 40 years

Subjects all fields of endocrinology (except diabetes mellitus)

Marius Tausk Career Development Award

Amount DM 15 000 —

Donator Organon G m b.H Munich

Applicants must reside in Europe and be not older than 33 years

Subjects, clinical and clinical-experimental endocrinology (except diabetes mellitus)

Applicants are invited to submit two copies of a previously unpublished paper in either German or English together with a short curriculum vitae and a description of the development of their scientific career to the President of the German Society for Endocrinology for 1973/74 Prof J R. Bierich M.D 74 Tübingen, Universitäts-Kinderklinik, Rümelinstraße 23 not later than October 15 1973

After receipt of the manuscript has been acknowledged by the German Society for Endocrinology the author is at liberty to have his paper published by a periodical.

Detailed information concerning the awards may be obtained from the President of the Society The awards will be presented at the 20th *Symposium of the German Society for Endocrinology* 1974

BOOKS RECEIVED

Nature's Transplant The transplantation immunology of viviparity By J Maxwell Anderson Butterworth London 1972. 145 p. £3 00

Interesting revue with an almost complete bibliography

Seek Wisely to Prevent Studies of attitudes and action in a cervical cytology programme. Edited by John Wakefield Department of Health and Social Security London Her Majesty's Stationary Office 1972. 193 p. £1 50

Recommended to organizers of cervical cytology programmes.

Diseases of the Vulva. By Nikolas A Janovski and Charles P Douglas. Harper & Row Publishers, Inc Hagerstown Maryland 1972. 1_5 p. 106 illustrations, 73 in color $17 50.

An excellent well illustrated small text book written by a pathologist and a gynaecologist as a team work. It is highly recommended to all obstetricians and gynaecologists.

I -S.

Acta Obstet Gynec Scand 52: 191–192, 1973

NEW INSTRUMENTS

THE ANKER DILATOR IN THERAPEUTIC ABORTION[1]

Bo von Friesen

From the Department of Obstetrics and Gynecology (Head: Bo von Friesen), Central Hospital, Lidköping, Sweden

In 1958, Herman Anker (1) described a new type of cervical dilator which was intended to supersede the instrument introduced by Lövset during the nineteen-thirties, as well as—to some extent—the Hegar dilator. The new dilator was simpler and no less effective; it had been used by Anker as a prelude to curettage in more than 800 cases.

The instrument is essentially a hairpin-shaped spring (Fig. 1). The two limbs, which are inserted into the uterus, are 7 cm long, transversely serrated on the outside and flat on the inside. When in apposition, the limbs are together equivalent in size to a no. 4 Hegar dilator. The proximal part of the instrument which lies in the vagina, is angled at 30° to the limbs; it is 9 cm long and is made of round steel of 3.5 mm diameter. The limbs are held in apposition by a retaining clip. When this is removed the limbs spring apart by up to 3 cm and can exert a pressure somewhat in excess of 1 kg.

We have used the Anker dilator as a prelude to the evacuation of early abortions since 1960. Before the hypertonic saline method came into general use, we also used the instrument as a prelude to partial evacuation. The instrument is normally inserted into the cervical canal on the afternoon of the preoperative day. When the retaining clip is removed, the patient feels a sensation of pressure but very seldom of slight pain. It is, in fact, remarkable how extremely little discomfort is experienced.

In 83% of 115 parous women, the patency achieved was sufficient to admit Saenger abortion forceps or a no. 14 (the largest) vacuum suction catheter, regardless of whether or not the pregnancy had progressed beyond the 12th week. In 46 nulliparous women the corresponding figure was 72%.

A vaginal pack was normally sufficient to prevent the instrument slipping out of place—which was one cause of failures. In 16 patients, this method permitted us to perform primary evacuation from the 13th up to the 16th week. There were no immediate complications attributable to the Anker dilator.

Adequate dilatation permits easy and safe curettage since big instruments can be used. It is vital, however, to know to what extent cervical damage may be inflicted by such dilatation.

In the literature, the author has been able to find only a few references to the incidence of cervical tears with Hegar dilators; two reports were from Germany. Lork (4) and Helbing (2) reported incidences of 2.4% and 4.2% respectively in connection with therapeutic abortion. No further details were available. An extensive study by Hulka & Higgins (3) revealed that the use of Hegar dilators prior to curettage frequently resulted in cervical lacerations; 154 such patients subsequently underwent hysterectomy and the cervices were carefully examined. The degree of dilatation performed was unfortunately not recorded.[2] In 39 ± 6.8% the internal os was torn to a depth of more than 2 mm and in 22 ± 6.8% to a depth of more than 5 mm. These tears were all of characteristic appearance

[1] In a personal communication, Professor A. Majewski of Hannover has disclosed that a similar dilator was introduced in Germany as long ago as the turn of the century. Majewski still uses this instrument, which he considers very useful in certain cases of abortion.

[2] The cervix was regularly dilated to Hegar 10–12 (J. F. Hulka, pers. com.)

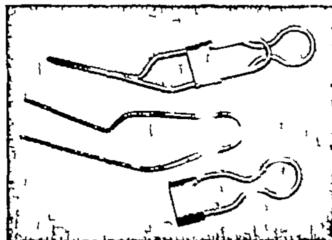


Fig 1 The Anker dilator

and they were invariably deepest at the level of the internal os. The immediate danger in these cases is parametrial infection and one of the later complications is incompetence of the internal os during pregnancy

FOLLOW UP STUDY

Of seventeen women giving birth after a history of previous therapeutic abortion in which the Anker dilator was used, four were primiparous and thirteen were multiparous. Pregnancy and delivery was normal in all except one of these. This latter patient who was primiparous, was delivered four weeks before term following premature rupture of the membranes. The infant weighed 3 180 g was healthy and showed no signs of prematurity It is uncertain whether or not this patient s cervix was incompetent

Hysterography was performed 2–4 months after therapeutic abortion in 11 patients, three of whom were nulliparous. The isthmus was normal with an intact surface and its maximum width in 7 patients was 4 mm in one patient 5 mm and in one patient 6 mm. In one patient (para II) the isthmus was described as uneven and its width as determined from a lateral view was 3 mm. In one further patient (para 0) the isthmus was ragged in appearance and 5–6 mm in width. Only in this latter patient does there seem to have been some evidence of injury

Discussion

Curettage or removal of products of conception is technically easier and less dangerous if the cervix is widely patent. Extensive dilation implies an obvious danger of tearing. Gradual dilatation could be expected to result in less damage than rapid dilation with Hegar dilators. All who are experienced in this field have felt on some occasion a sudden loss of resistance at some stage during dilatation with Hegar dilators. This can only signify that the cervix has been torn. It seems unlikely that dilatation to Hegar no. 10 or more can be performed without a high incidence of cervical lacerations. A follow-up study of patients on whom Hegar dilators have been used would provide valuable information in this respect.

The Anker dilator provides slow but effective dilatation. The scale of our study is small but the results nevertheless suggest that the method is safe The author and his colleagues have been impressed by the simplicity and effectiveness of the instrument.

The instrument was originally manufactured in Norway but those in use at our clinic are replicas made for us by the hospital engineering department.

ACKNOWLEDGEMENT

Our thanks are due to Dr Janos Schvarcz for interpretation of the X-rays.

REFERENCES

1 Anker H. Norsk Lægeforen 78 509 1958.
2 Helbing, W Zbl Gynäk 45 2008, 1954.
3. Hulka, J F & Higgins, G. Amer J Obst Gynec 6. 913 1961
4 Lork, E C., Geburtsh Frauenheilk 8. 493 1948

Submitted fo publication March 20 1972

Bo von Friesen
Fri Kaflet 1
S-531 00 Lidköping
Sweden

INTRA UTERINE FETAL GROWTH STUDIED BY ULTRASONIC BIPARIETAL MEASUREMENTS

The Percentiles of Biparietal Distribution

S. Levi and P. Smets

From the Clinique Obstétricale et Gynécologique (Head: Professor R. Vokaer), Hôpital Universitaire Brugmann, Université Libre de Bruxelles, Brussels, Belgium

Abstract. Fetal biparietal diameter was measured in utero with ultrasonic techniques, in 1011 patients with 3032 measurements at gestational ages ranging from 15 to 43 weeks. The distribution of these values, the mean weekly increments, together with the 5th to 95th percentiles are presented in tabular and graphic form. The mean values correspond to those quoted in other investigations.

Fetal growth has been studied by radiological and ultrasonic methods for many years. Measurement of fetal biparietal diameter (B.P.D.) during pregnancy is commonly used in fetal growth studies (2, 3, 6, 8, 11, 12, 13, 14, 15, 16).

The mean values for B.P.D. published previously are similar with the exception of the observations of Campbell (3) whose B.P.D. values are somewhat higher (Fig. 1).

Abnormal fetal growth should correlate with abnormal B.P.D. growth. In those studies based on reference to an average growth curve (1, 4, 7, 9, 10, 17) the results were not always very conclusive. We consequently think that the range of B.P.D. expressed for every stage of gestation would be useful for clinical purposes.

As far as we know such study has not been made with specific reference to *in utero* B.P.D. measurements on healthy fetuses from normal pregnant women in normal pregnancy conditions. Campbell & Newmann (5) were concerned with a particular and very important aspect of this problem when they studied the B.P.D. growth variations with the percentile method.

MATERIALS AND METHODS

We started to measure the B.P.D. in 1966, and since 1969 more than 95% of the patients attending our antenatal clinic have been scanned by ultrasound (Aloka equipment, Japan Radio Co., Tokyo).

The B.P.D. was measured during pregnancy by means of two different ultrasonic procedures: first, the fetal head is localized by the B-scan method on two-dimensional display (Fig. 2), then the sonic probe is maintained exactly in the position where the ultrasonic beam is perpendicular to the parietal bones as well as to the midline echo, which probably corresponds to the third cerebral ventricle or to the interhemispheric groove.

In such circumstances the distance between the two parietal protuberances—the B.P.D.—can be read directly on the A-scan screen, with an electronic scale calibrated at a speed of 1529 m/sec, considered as the average velocity of sounds in human body (Fig. 3).

Usually the average of three different readings is taken. The standard deviation for a single reading is 1.2 mm (computed from 15 cases with 10 duplicate readings in each case, at gestational ages ranging between 16 and 42 weeks and B.P.D. values ranging between 36 and 97 mm). The homogeneity of the Variance was tested and not rejected. Therefore we assumed that the variance of the measurement was identical for every B.P.D.

The data of 1671 patients were recorded on IBM cards. We excluded from this study the B.P.D. obtained in toxemic (E.P.H.—Gestosis) and diabetic patients, when pregnancy age was doubtful or unknown, in cases of Rh-immunization and multiple pregnancies, or when placental insufficiency was diagnosed. 1011 cases were used for computation involving 3032 B.P.D. measurements.

The gestational age is expressed in completed weeks, calculated from the first day of the last menstrual period.

The 5th, 10th, 20th, 50th, 80th, 90th, 95th, percentiles of the distribution of B.P.D. for each week—from 15 to 43—of gestation were computed from 3032 B.P.D. meas-

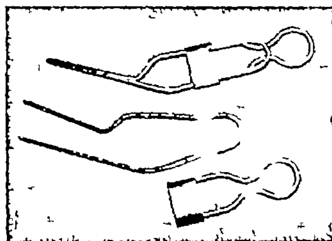


Fig 1 The Anker dilator

and they were invariably deepest at the level of the internal os. The immediate danger in these cases is parametrial infection and one of the later complications is incompetence of the internal os during pregnancy

FOLLOW UP STUDY

Of seventeen women giving birth after a history of previous therapeutic abortion in which the Anker dilator was used, four were primiparous and thirteen were multiparous. Pregnancy and delivery was normal in all except one of these. This latter patient, who was primiparous, was delivered four weeks before term following premature rupture of the membranes. The infant weighed 3 190 g was healthy and showed no signs of prematurity It is uncertain whether or not this patient s cervix was incompetent

Hysterography was performed –4 months after therapeutic abortion in 11 patients, three of whom were nulliparous. The isthmus was normal, with an intact surface and its maximum width in 7 patients was 4 mm, in one patient 5 mm and in one patient 6 mm. In one patient (para II) the isthmus was described as uneven and its width as determined from a lateral view was 3 mm. In one further patient (para 0) the isthmus was ragged in appearance and 5–6 mm in width. Only in this latter patient does there seem to have been some evidence of injury

Discussion

Curettage or removal of products of conception is technically easier and less dangerous if the cervix is widely patent. Extensive dilation implies an obvious danger of tearing. Gradual dilatation could be expected to result in less damage than rapid dilation with Hegar dilators. All who are experienced in this field have felt on some occasion a sudden loss of resistance at some stage during dilatation with Hegar dilators. This can only signify that the cervix has been torn. It seems unlikely that dilatation to Hegar no. 10 or more can be performed without a high incidence of cervical lacerations. A follow-up study of patients on whom Hegar dilators have been used would provide valuable information in this respect.

The Anker dilator provides slow but effective dilatation. The scale of our study is small but the results nevertheless suggest that the method is safe. The author and his colleagues have been impressed by the simplicity and effectiveness of the instrument.

The instrument was originally manufactured in Norway but those in use at our clinic are replicas made for us by the hospital engineering department.

ACKNOWLEDGEMENT

Our thanks are due to Dr Janos Schwarcz for interpretation of the X-rays.

REFERENCES

1 Anker H Nord Lægeforen 8 509 1958.
2 Helbing, W Zbl Gynäk 45 2008 1954.
3. Hulka, J F & Higgins, G Amer J Obst Gynec 6. 913 1961
4 Lork, E C Geburtsh Frauenheilk 8 493, 1948.

Submitted for publication March 20 1972

Bo von Friesen
Erl Kafles 1
S- 31 00 Lidköping
Sweden

Acta Obstet Gynec Scand 52. 193–198 1973

INTRA UTERINE FETAL GROWTH STUDIED BY ULTRASONIC BIPARIETAL MEASUREMENTS

The Percentiles of Biparietal Distribution

S. Levi and P. Smets

From the Clinique Obstétricale et Gynécologique (Head: Professor R. Vokaer), Hôpital Universitaire Brugmann, Université Libre de Bruxelles, Brussels, Belgium

Abstract. Fetal biparietal diameter was measured in utero with ultrasonic techniques, in 1 011 patients with 3 032 measurements at gestational ages ranging from 15 to 43 weeks. The distribution of these values, the mean weekly increments, together with the 5th to 95th percentiles are presented in tabular and graphic forms. The mean values correspond to those quoted in other investigations.

Fetal growth has been studied by radiological and ultrasonic methods for many years. Measurement of fetal biparietal diameter (B.P.D.) during pregnancy is commonly used in fetal growth studies (2, 3, 6, 8, 11, 12, 13, 14, 15, 16).

The mean values for B.P.D. published previously are similar with the exception of the observations of Campbell (3) whose B.P.D. values are somewhat higher (Fig. 1).

Abnormal fetal growth should correlate with abnormal B.P.D. growth. In those studies based on reference to an average growth curve (1, 4, 7, 9, 10, 17), the results were not always very conclusive. We consequently think that the range of B.P.D. expressed for every stage of gestation would be useful for clinical purposes.

As far as we know such study has not been made with specific reference to *in utero* B.P.D. measurements on healthy fetuses from normal pregnant women in normal pregnancy conditions. Campbell & Newmann (5) were concerned with a particular and very important aspect of this problem when they studied the B.P.D. growth variations with the percentile method.

MATERIALS AND METHODS

We started to measure the B.P.D. in 1966, and since 1969 more than 98% of the patients attending our antenatal clinic have been scanned by ultrasound (Aloka equipment, Japan Radio Co., Tokyo).

The B.P.D. was measured during pregnancy by means of two different ultrasonic procedures: first, the fetal head is localized by the B-Scan method on a two-dimensional display (Fig. 2), then the sonic probe is maintained exactly in the position where the ultrasonic beam is perpendicular to the parietal bones as well as to the midline echo, which probably corresponds to the third cerebral ventricle or to the interhemispheric groove.

In such circumstances the distance between the two parietal protuberances—the B.P.D.—can be read directly on the A-Scan screen, with an electronic scale calibrated at a speed of 1 529 m/sec, considered as the average velocity of sounds in human body (Fig. 3).

Usually the average of three different readings is taken. The standard deviation for a single reading is 1.2 mm (computed from 15 cases with 10 duplicate readings in each case, at gestational ages ranging between 16 and 42 weeks and B.P.D. values ranging between 36 and 97 mm). The homogeneity of the Variance was tested and not rejected. Therefore we assumed that the variance of the measurement was identical for every B.P.D.

The data of 1 671 patients were recorded on IBM cards. We excluded from this study the B.P.D. obtained in toxemic (E.P.H.—Gestosis) and diabetic patients, when pregnancy age was doubtful or unknown, in cases of Rh-immunisation and multiple pregnancies, or when placental insufficiency was diagnosed. 1 011 cases were used for computation involving 3 032 B.P.D. measurements.

The gestational age is expressed in completed weeks, calculated from the first day of the last menstrual period.

The 5th, 10th, 20th, 50th, 80th, 90th, 95th, percentiles of the distribution of B.P.D. for each week—from 15 to 43—of gestation are computed from 3 032 B.P.D. mea-

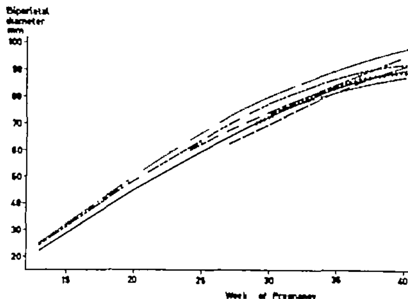


Fig 1 Fetal biparietal diameter mean values. Brown (2) – – Campbell (3) Hinselmann (8) —— Kratochwil (11) + + Levi (12) – – Ojala (13) – Scammon (X-rays) (14) ——

urements. When the number of cases is too small (less than 36) some percentiles are omitted as being not reliable

To compute the growth rate at various gestation age, we use the same techniques as Campbell & Newmann (5). For each patient, the growth rate was considered as the difference between two successive measurements divided by the intervening time interval and the computed growth rate was assumed to be that for the mid-point of this period.

For each patient, the growth rates were estimated. (*a*) by using neighbouring pairs of points, or (*b*) by using all possible pairs of points.
No significant difference was observed between the results obtained with the two methods, this was also noted by Campbell & Newmann (5) Consequently we used all possible pairs.

For the graphical representation only we smoothed the date obtained weekly with a weighted moving average based on 7 points for the fetal B.P.D (Fig. 4) and the percentiles of its growth rate on 5 points (Fig. 5) The weights are proportional to the number of observations. For instance, the percentiles of the fetal B P.D at week 30 are estimated with all diameters obtained from week 27 to week 33 and the growth rate at week 30 is estimated with all growth rates obtained from week 28 to week 32.

RESULTS

Table I presents the number of measurements, the mean the standard deviation and the 5th to 95th percentiles of the B P D distribution from the 15th to the 43rd week of gestation and Fig. 4 presents the smoothed percentiles of this distribution

Table II presents the mean the standard deviation and the 10th to 90th precentiles of the weekly growth of the B P D for each week of pregnancy from the 15th to the 43rd and Fig. 5 presents the smoothed percentiles of this distribution Fig. 6 shows the mean growth rate and 1 standard deviation.

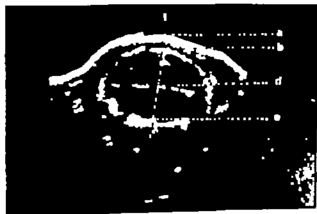


Fig 2 Fetal head cross sectional display on ultrasonic B-Scan screen (*a*, *b* *d* see Fig. 3).



Fig 3 Fetal biparietal diameter measurement on ultrasonic A-Scan screen. BP value on electronic scale: 76 mm ( ) maternal abdominal wall; (*b*) upper fetal parietal bone (*d*) midline echo ( ) lower fetal parietal bone (*f*) maternal sacrum.

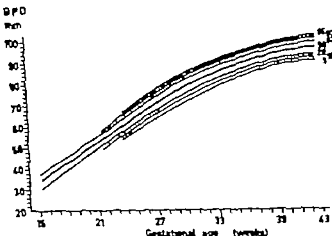


Fig. 4. Percentiles of biparietal diameter distribution (smoothed curves).

DISCUSSION

The concentration of the distribution of B.P.D values does not vary very much according to the gestation period. That concentration is not more important t the beginning of pregnancy than at the end

Here is a possible explanation. at the end of pregnancy the concentration of the distribution is most probably influenced by nutritional or genetic factors It is less important when B P D is concerned than for weight (variation coefficient, 13 % mean, 3 340 g S.D 435 g), and similar as for height (variation coefficient, 3.3 % mean, 49 7 cm S D 1.6 cm) the B P.D variation coefficient being 3 7 % (mean, 94 mm, S.D 3.5 mm).

At the beginning of pregnancy a more concentrated B.P D distribution should be expected as environment has had no time yet to exert all its action. And yet, on each side of the man, the B.P D distribution range between percentiles 20 and 80 is respectively of 6 2 and 6 mm at weeks 15 and 40.

Two explanations are possible: (i) the hardly avoidable mistakes about the estimation of ovular age are more disturbing when weekly growth rate is very high (3 88 mm per week at week 15 as opposed to 1 35 mm at week 40) (ii) the errors

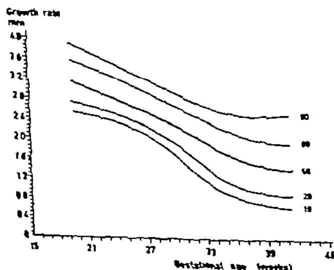


Fig. 5. Percentiles of biparietal diameter, increments distribution (smoothed curves).

Table I *Distribution of the fetal biparietal diameter 5th to 95th percentiles*

Gestation age (weeks)	Mean (mm)	S.D	Percentiles of the biparietal diameter 5%	10%	20%	50%	80%	90%	95%	No. of measurements
15	32.0	3.0	—	—	29 6	31 7	35.8	—	—	18
16	35.5	3 4	—	—	32.4	35.6	38.2	—	—	19
17	38.9	2.9	—	—	35 8	39 7	41.8	—	—	27
18	41.2	3.6	—	—	38.0	41 7	44.2	—	—	25
19	44.3	2.7	—	—	41.5	44.4	46.3	—	—	35
20	47 6	2.6	—	—	45 6	47.9	49.9	—	—	26
21	50.0	3 7	—	—	47.2	49.9	52.3	—	—	24
22	53.5	3 4	48.4	49 7	50.2	53 8	56.1	58.0	59.5	39
23	56.9	3 6	—	51.8	53.8	57 7	59.9	60.3	—	34
24	59 4	3.9	53.7	54.1	55.9	59 6	62.4	64 7	66.0	48
25	63 1	3 6	56.8	58.3	60.0	63.7	65.6	66.4	69.8	47
26	65.5	4.8	59.5	60.2	62.1	65.7	69 6	70.3	74.0	41
27	68.3	5.1	62.2	63 9	65 4	68.1	72.2	74.2	75.6	54
28	71.4	4.2	61.9	64 4	69 6	72.0	74.2	75.9	77 6	58
29	74.7	4 4	67 4	69.9	71 8	74 4	77 7	80.1	8[illegible].6	79
30	77.5	4 6	69.8	71.5	73.9	77 7	81 7	83 8	84.3	81
31	79.8	3.9	72.3	74.2	76.4	80.0	83.6	84.3	85 7	90
32	81.5	4.3	74.3	76.3	78 4	81 7	85 7	86.2	86.4	125
33	83.8	3.8	77.9	87.9	81 6	83.9	86.3	87.9	89 9	132
34	85.1	3.7	79.5	80.0	82.4	85.6	87.9	89 6	90.3	145
35	86.7	3.9	79 9	81 7	84 0	86.4	89 9	91 7	92.5	167
36	88.5	3.6	82.0	83.6	85.7	88.3	91.8	93 0	94.0	200
37	90.3	3.3	85.6	86.0	87 6	90.1	93.4	94.5	95.8	258
38	91 7	3.1	86.6	87.8	89.3	91.8	94.4	96.0	96.8	334
39	92.8	3.2	87 6	88 9	90.2	92.7	95.5	96.5	98.0	335
40	94 1	3.5	87 7	89 7	90.9	94.2	96.9	98.5	100.0	297
41	94 4	3 4	89 6	90.1	91.5	94 7	97.3	98.7	93.6	171
42	94.9	4.1	89.5	90.4	91 4	95.0	98.3	100.0	101.0	83
43	94.8	4.0	—	—	91.5	95.5	98.5	—	—	20
										3 01.

on the readings are similar when B P.D are small or large but are relatively more important in be ginning of pregnancy i e. 2 mm error when B P D is equal to 32 mm corresponds to 6% and only 2% when B.P.D is equal to 94 mm.

Finally let us note that the difference between B P.D averages reported by different authors, in particular by Campbell (3) and ourselves, could be explained by this fact the propagation velocity of ultrasounds in tissues is differently estimated

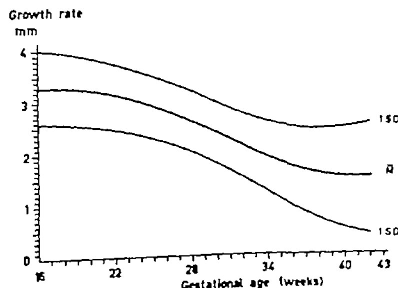


Fig 6 Biparietal diameter increments, mean plus 1 S D

Table II. *Distribution of the fetal biparietal diameter growth rate, 10th to 90th percentiles*

Gestation age (weeks)	Mean (mm)	S.D.	Percentiles of growth 10%	20%	50%	80%	90%	No. of pairs
15	3.30	0.57	—	—	3.88	—	—	5
16	3.24	0.46	—	—	3.20	—	—	8
17	3.15	0.54	—	2.56	3.20	3.44	—	11
18	3.09	1.10	—	2.85	3.05	3.85	—	11
19	3.48	0.89	—	2.80	3.25	3.90	—	19
20	3.17	0.40	2.56	2.69	3.15	3.33	3.63	22
21	3.19	0.90	2.56	2.70	3.10	3.70	3.89	33
22	3.22	0.52	2.50	2.75	3.08	3.43	3.90	31
23	3.00	0.42	2.31	2.58	2.85	3.31	3.49	42
24	3.02	0.68	2.32	2.56	2.85	3.20	3.47	49
25	3.01	0.64	2.41	2.55	2.74	3.06	3.36	81
26	2.78	0.46	2.26	2.54	2.58	3.01	3.21	91
27	2.73	0.57	2.16	2.33	2.37	2.97	3.22	125
28	2.63	0.55	2.12	2.23	2.47	2.76	3.06	149
29	2.59	0.59	2.06	2.17	2.42	2.68	2.93	154
30	2.57	0.67	1.86	2.00	2.30	2.64	2.90	195
31	2.25	0.51	1.69	1.87	2.16	2.42	2.68	214
32	2.14	0.48	1.56	1.78	2.02	2.37	2.81	224
33	2.03	0.47	1.17	1.45	1.92	2.32	2.56	283
34	1.94	0.63	1.15	1.39	1.85	2.23	2.60	322
35	1.79	0.66	0.95	1.21	1.67	1.85	2.25	361
36	1.70	0.81	0.90	1.10	1.54	1.93	2.44	397
37	1.55	0.75	0.76	0.91	1.40	1.91	2.25	395
38	1.55	0.91	0.69	0.89	1.45	1.92	2.36	385
39	1.46	0.93	0.43	0.85	1.38	1.94	2.88	322
40	1.36	1.23	0.57	0.87	1.38	2.35	2.91	397
41	1.67	1.28	0.63	0.87	1.37	2.34	2.93	94
42	1.38	0.97	0.62	0.87	1.34	1.91	1.93	40
43	0.89	0.60	—	—	—	—	—	9

and consequently the constant to convert the time intervals between 2 successive echoes into distances is different as well: for instance at week 40 the ratio of average B.P.D. to the sound velocity chosen as constants are respectively for Campbell and ourselves.

97 mm/94 mm = 1.032, and
1 600 m per sec/1 529 m per sec = 1.046.

Moreover Campbell & Newmann have voluntarily rejected measurements of children born with a weight inferior to 5th percentile and this has probably contributed to increase their B.P.D. average.

CONCLUSIONS

The interest of this work is mainly clinical: the B.P.D. values obtained by ultrasonic measurements, at *different* pregnancy ages may be plotted on the graphs and their situation considered *for* clinical use. If the different successive B.P.D. values are regularly reported on a given percentile curve, the growth can be considered as satisfactory. If the individual curve is different from the reference one, a conclusion of insufficient or excessive growth can be *inferred* (hypotrophy or microcephaly, macrosomia or hydrocephaly).

When the gestation age is unknown, a first B.P.D. measurement, performed as early as possible in pregnancy, makes it possible to give a rather accurate fetus age and then watch the growth from the growth rate curves.

REFERENCES

1. Boog, G., Irmann, M., Muller, O. & Gandar, R.: *Céphalométrie foetale par ultrasons. III. Dépistage de la souffrance foetale chronique.* Rev Franç Gynéc 64: 315–320, 1969.
2. Brown, R. E.: Ultrasound fetal cephalometry. J Ultrasonographica Medica, vol. III, pp. 193–198. Verlag der Wiener Medizinischen Akademie, Wien, 1971.
3. Campbell, S.: An improved method of fetal cephalometry by ultrasound. J Obstet Gynaec Brit Cwlth 75: 568–576, 1968.

Table I *Distribution of the fetal biparietal diameter 5th to 95th percentiles*

Gestation age (weeks)	Mean (mm)	S D	Percentiles of the biparietal diameter 5%	10%	20%	50%	80%	90%	95%	No. of measurements
15	32.0	3.0	—	—	29.6	31.7	35.8	—	—	18
16	35.5	3.4	—	—	32.4	35.6	38.2	—	—	19
17	38.9	2.9	—	—	35.8	39.7	41.8	—	—	27
18	41.2	3.6	—	—	38.0	41.7	44.2	—	—	25
19	44.3	2.7	—	—	41.5	44.4	46.3	—	—	35
20	47.6	2.6	—	—	45.6	47.9	49.9	—	—	26
21	50.0	3.7	—	—	47.2	49.9	52.3	—	—	4
22	53.5	3.4	48.4	49.7	50.2	53.8	56.1	58.0	59.5	39
23	56.9	3.6	—	51.8	53.8	57.7	59.9	60.3	—	34
24	59.4	3.9	53.7	54.1	55.9	59.6	62.4	64.7	66.0	43
25	63.1	3.6	56.8	58.3	60.0	63.7	65.6	66.4	69.8	47
26	65.5	4.8	59.5	60.2	62.1	65.7	69.6	70.3	74.0	41
27	68.3	5.1	62.2	63.9	65.4	68.1	72.2	74.2	75.6	54
28	71.4	4.2	61.9	64.4	69.6	72.0	74.2	75.9	77.6	58
29	74.7	4.4	67.4	69.9	71.8	74.4	77.7	80.1	82.6	79
30	77.5	4.6	69.8	71.5	73.9	77.7	81.7	83.8	84.3	81
31	79.8	3.9	72.3	74.2	76.4	80.0	83.6	84.3	85.7	90
32	81.5	4.3	74.3	76.3	78.4	81.7	85.7	86.2	86.4	125
33	83.8	3.8	77.9	87.9	81.6	83.9	86.3	87.9	89.9	132
34	85.1	3.7	79.5	80.0	82.4	85.6	87.9	89.6	90.3	145
35	86.7	3.9	79.9	81.7	84.0	86.4	89.9	91.7	92.5	167
36	88.5	3.6	82.0	83.6	85.7	88.3	91.8	93.0	94.0	200
37	90.3	3.3	85.6	86.0	87.6	90.1	93.4	94.5	95.8	258
38	91.7	3.1	86.6	87.8	89.3	91.8	94.4	96.0	96.8	334
39	92.8	3.2	87.6	88.9	90.2	92.7	95.5	96.5	98.0	355
40	94.1	3.5	87.7	89.7	90.9	94.2	96.9	98.5	100.0	297
41	94.4	3.4	89.6	90.1	91.5	94.7	97.3	98.7	93.6	171
42	94.9	4.1	89.5	90.4	91.4	95.0	98.3	100.0	101.0	83
43	94.8	4.0	—	—	91.5	95.5	98.5	—	—	70
										3 03

on the readings are similar when B.P D are small or large but are relatively more important in beginning of pregnancy i.e. 2 mm error when B.P D is equal to 32 mm corresponds to 6% and only 2% when B.P D is equal to 94 mm.

Finally let us note that the difference between B.P D averages reported by different authors, in particular by Campbell (3) and ourselves, could be explained by this fact the propagation velocity of ultrasounds in tissues is differently estimated

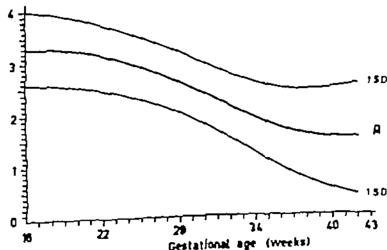


Fig 6 Biparietal diameter increments, mean plus 1 S D.

Acta Obstet Gynec Scand 52: 199–204 1973

CALF BLOOD FLOW DURING NORMAL PRIMIPREGNANCY

Bo Sandström

From the Department of Obstetrics and Gynecology (Head, Professor Per Lundström) and the Department of Clinical Physiology (Head: Professor Håkan Linderholm), University of Umeå, Sweden

Abstract. The blood flow in the lower legs of 64 primigravidae was examined plethysmographically by which measures of mainly the muscle flow was obtained. The subjects were divided into three groups according to duration of pregnancy and the blood flow at rest and during reactive hyperaemia was recorded. The periods of pregnancy were 9–13 weeks (Group A), 15–22 weeks (Group B) and 37–42 weeks (Group C). Subjects in Group A were examined 1 day, 2 weeks and in a few cases 6 weeks after abortion in addition to examination during pregnancy; subjects in Groups B and C were examined 2 weeks after abortion and delivery respectively. The blood flow at rest during pregnancy expressed in ml/min 100 ml tissue was lower in Group C than in Groups A and B. During reactive hyperaemia the blood flow did not differ significantly between the three groups. In early pregnancy the muscular blood flow does not seem to increase in comparison with the nonpregnant state. The increased cardiac output during early pregnancy thus does not seem to pass through the muscles of the limbs. During the latter half of pregnancy resting blood flow measured with the subject in the supine position decreases, which is contrary to earlier reports.

Most determinations of peripheral blood flow have been performed with the aid of venous occlusion plethysmography. Earlier studies of the peripheral circulation in the legs and the arms during pregnancy gave divergent results (1, 3, 10, 11, 15, 16, 20, 21, 24, 25).

The lower leg blood flow at rest has been variously reported as unchanged (1, 10, 11) and increased (15, 16). In the forearm the blood flow is reported as unchanged (1, 10) and increased (3, 11, 15, 16, 24, 25). All these results were obtained during the latter half of pregnancy.

Spetz (24) investigated blood flows in the forearm. During early pregnancy (11th–13th week), the flow did not differ significantly from that in nonpregnancy either at rest or during reactive hyperaemia. However, from the middle of pregnancy there was an increase in both rates of flow. Lower peripheral vascular resistance was considered to be coincident with the increase in the rate of flow. Employing the ^{133}Xe clearance method, Spetz & Jansson (25) later demonstrated that the increase of flow in the forearm during the latter half of pregnancy is exclusively due to increased skin flow, with the skeletal muscle flow remaining constant.

In a preliminary report MacGregor & Snodgrass (20) demonstrated a reduction in the resting forearm blood flow of 95 gravidae during early pregnancy, but the blood flow increased as pregnancy progressed. The investigators report an increase in the peripheral vascular resistance during early pregnancy. The results have to be regarded against the background of an increased cardiac output (18).

The case materials referred to above consist of both primigravidae and multigravidae, and the description of the methodology is often incomplete. No examinations of blood flow through the lower legs were performed prior to the 12th week of pregnancy and no examinations during reactive hyperaemia in the lower legs were made in primigravidae. In view of these facts and the divergent results reported in the literature, blood flow determinations in the present study were performed in uniform and distinctly defined groups. Primigravidae were chosen in order to eliminate any effect that previous pregnancies might have on the peripheral circulation of the legs.

4 Campbell, S. & Dewhurst, C. J Diagnosis of the small-for-dates fetus by serial ultrasonic cephalometry Lancet *II* 1002–1006 1971
5 Campbell, S. & Newman, G B. Growth of the fetal biparietal diameter during normal pregnancy J Obstet Gynaec Brit Comm *78* 513–519 1971
6. Garrett, W J & Robinson, D E. Ultrasound in Clinical Obstetrics. C. C. Thomas (ed.), Springfield, Illinois, 1970.
7 Hibbard, L. T & Anderson, G. V Clinical applications of ultrasonic fetal cephalometry Obstet Gynec (N Y) *29* 84–847 1967
8. Hinselmann, M., Ergebnisse der routinemäßigen Kephalometrie in der Schwangerenvorsorge *In* Ultrasonographia Medica, vol. III pp. 207–211 Verlag der Wiener Medizinischen Akademie Wien, 1971
9 Hollander H. J Die Ultraschalluntersuchung im Rahmen der pränatalen Diagnostik der Rh-Erythroblastose *In* Ultrasonographia Medica, vol. III, pp. 227–234. Verlag der Wiener Medizinischen Akademie Wien, 1971
10. Jouppila, P., Ylostalo, P & Pystynen, P Fetal head growth measured by ultrasound in the last few weeks of pregnancy in normal, toxaemic and diabetic women. Acta Obstet Gynec Scand *49* 367 369 1970.
11 Kratochwil, A. Ultraschalldiagnostik in Geburtshilfe und Gynäkologie. G Thieme Verlag, Stuttgart, 1968
12. Levi, S. Ultrasonodiagnostic en obstétrique: Intérêt clinique de la mesure du diamètre bipariétal du foetus. *Gyn Obstét (Paris) 69* 227–38 1970.
13 Ojala, A., Ylöstalo, P., Jouppila, P & Järvinen, P A. Der biparietale Durchmesser bei normalen Schwangerschaftsstörungen. *In* Ultrasonographia Medica, vol. III, pp. 199–205. Verlag der Medizinischen Akademie, Wien, 1971
14 Scammon, R. E. & Calkins, L. A. The Development and Growth of the External Dimensions of the Human Body in the Fetal Period. University of Minnesota Press, Minneapolis, 1929
15 Thompson, H. E. Studies of fetal growth by ultrasound. *In* Diagnostic Ultrasound, pp. 416–4?7 Plenum Press, New York, 1966.
17 Willocks, J Donald, I., Campbell, S & Dunsmore, I. R., Intrauterine growth assessed by ultrasonic foetal cephalometry J Obstet Gynaec Brit Comm *74* 639–647 1967
16. Willocks, J Donald, I., Duggan, T C. & Day N. Foetal cephalometry by ultrasound. J Obstet Gynaec Brit Comm *71* 11 20, 1964

S bmitted for publication February 7 197

Salvator Levi
Clinique Obstétricale et Gynécologique
Hôpital Universitaire Brugmann
Place van Gehuchten 4
1020 Brussels
Belgium

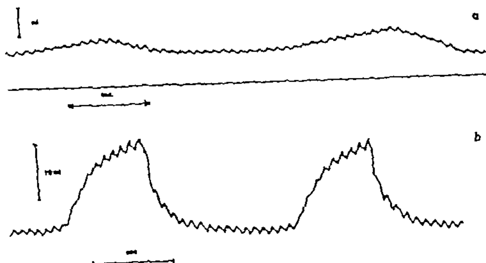


Fig. 2. Blood flow curves (*a*) at rest, (*b*) during reactive hyperaemia.

The apparatus was calibrated before and after each examination in the following manner. Ten ml of water at 37°C were injected into the plethysmograph and evacuated by suction, during which the deflection of the recorder pointer was noted and the calibration constant could be determined in mm/ml. By measuring the amount of water in the plethysmograph at the end of the examination, the volume of the calf segment could be calculated. For further details about the apparatus see Dahn (6).

Procedure. The examinations were performed in the afternoon (1–3 p.m.) with the patient in the supine position and with both legs placed in the plethysmograph (Fig. 1). In order to reach a state as basal as possible, 5 mg of diazepam were administered orally two hours before the examination. In the dose administered the drug is considered not to affect the circulation (23). The lower legs were positioned at a level of midthoracic height, i.e. approximately at the level of the right atrium. The room was quiet and the ambient temperature 22°C.

After the patient had lain with her lower legs enclosed in the plethysmographs for approximately 20 min, the arterial occlusion cuffs were applied at a pressure of 250 mmHg around both ankles. About one minute later venous occlusion cuffs were applied to each thigh just above the knee joints and six recordings were made. An occluding pressure of 55 mmHg was used.

Reactive hyperaemia was then induced by arterial occlusion in the thigh at a pressure of 250 mmHg for 5 min in combination with muscular exercise on the Thulesius foot ergometer (27) during the first 4 min.

The arterial occlusion cuff at the ankle was then re-applied and the occluding pressure on the thigh was released after reapplication. Recordings were made on the kymograph after applying the venous occlusion cuff to the thigh. In this manner recordings were made of the first flow of the peak flow—which, on the average, appeared 30 sec after releasing the arterial occlusion pressure—and of additional flows at intervals of approximately 15 sec during 3–5 min of the period of reactive hyperaemia. After this period the rates of flow had generally reached the magnitude prevailing at rest. Plethysmograms at rest and during reactive hyperaemia are illustrated in Fig. 2.

Calculations. The blood flow rates were calculated from the plethysmograms and the slope of the volume curve in relation to the time axis was determined. In case the increasing volume was not linear during the entire period of venous occlusion, the measurement of the slope was estimated during the first 3–4 pulse beats. The flow was calculated from the following formula: $F = (1\,000 \cdot h)/(c \cdot V)$ where F is the flow in ml/min · 100 ml tissue, h is the extrapolated perpendicular rise of the flow curve slope in 6 seconds, c is the calibration constant in mm/ml, and V is the volume of the leg segment in ml obtained by subtracting the drained volume of water from the volume of the plethysmograph (3 470 ml) at the end of the examination.

The error of a single measurement was calculated from six double determinations on two consecutive days and was found to be 19% of the mean in determination of the peak blood flow and 12% of the mean in determination of the resting blood flow. The error of measurement on the same day was calculated from twelve double determinations and was found to be 10% of the mean in determination of the resting blood flow. The calculations of the methodological error were performed according to Enghoff (8).

Determination of haemoglobin concentration

The haemoglobin concentration was determined in peripheral blood at the time of the plethysmographic examination. The determinations were performed spectrophotometrically at a wavelength of 544 mμ (oxyhaemo-

Table I *Age menarche duration of pregnancy and haemoglobin concentration in Groups A, B and C*

Mean ± S.D Ranges in brackets

	Group A (-27)	Group B (n-16)	Group C (-.5)
Age, years	18 7±3.2 (15-27)	19.6±4 4 (15-32)	.3.1±3.2 (19-30)
Menarche, years	12.9±1 1 (11-15)	13.1±1 1 (11-15)	—
Duration of pregnancy weeks	12.0±1 1 (9-13)	17.3±1.9 (15-22)	38.2±1.3 (37-42)
Haemoglobin, g% (pregnancy)	11 4±0 8 (10.1-13.1)	10.8±0.9 (9.3-12.2)	11 6±0.6 (10.5-13.2)
Haemoglobin, g% (after pregnancy)	12.0±0.8 (10.6-13.4)	11 6±0.7 (10.3-1..4)	12.9±0.8 (11.2-15.2)

MATERIAL

The series consisted of 68 healthy primipregnant volunteers constituting three groups designated as Group A (9-13 weeks), Group B (15-22 weeks) and Group C (37-42 weeks).

In Group A and Group B legal abortion was granted on psychiatric or sociomedical grounds and performed by vacuum aspiration (17) and by saline insufflation methods respectively The patients in Group C were recruited at the prenatal clinic about 1 month before the calculated date of deli ery Additional data are given in Table I.

The first examination was performed during the pregnant state in all three groups and the second one weeks after abortion (Group A and Group B) or delivery (Group C) One day after abortion an additional xamination was performed in Group A.

Before the study was started the patients were told how the trial was to be conducted and gave their full consent.

METHODS

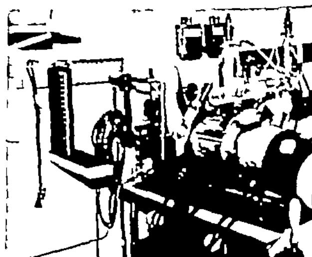
Venous occlusion plethysmography (VOP)

A number of methods are available for determining the peripheral circulation f the legs The advantages nd disadvantages of the different methods ha e been discussed by for instance Greenfield et l. (14), Dahn, (7) Kappert (19) and Bollinger (2)

Theory Sudden occlusion of the enous return from an extremity results in a progressive increase in the olume of the limb segment distal to the occlusion due to an accumulation of blood in the vascular bed. The increase in volume after blocking the venous return may be dete mined by measuring, for instance the amount of displaced water in the plethysmograph. It is essential that the eins should be emptied complet ly between any two measurements. Consequently the blood flow can only be deter mined in the horizontal position or with the limb levated.

All types of oedema in the extremities prevent reliable blood flow determinations using VOP (2). This is because VOP utilizes the increase in volume of the tissues after subdiastolic stasis as a measure of the arterial inflow and local oedema would interfere with the changes in volume following venous occlusion.

Apparatus. Since none of the available plethysmographic methods satisfy all requirements with respect to the evaluation of the peripheral blood flow in the legs, use made of water-filled plethysmographs. The reproducibility of this method is good and it provides a means for determining the venous volume and capillary filtration, thus facilitating comparisons with earlier investigations (6, 13, .3). By using an expansion chamber with a large cross-section (5), pressure variations in the plethysmograph were practically eliminated. The water temperature of the plethysmograph was kept constant at 37°C, and the mean pressure exerted by the water on the lower leg 10 mmHg. The temperature of 37°C was chosen to make possible comparisons with other blood flow measurements in early pregnancy (?4). The volume of the plethysmographs was 3 470 ml each. The time was marked at every sixth second at the bottom of the plethysmogram.



a



b

Fig 1 The arrangement of the plethysmographs and foot ergometers. For details see Dahn, 1965 (6).

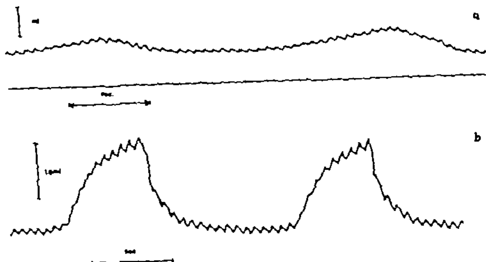


Fig. 2. Blood flow curves (*a*) at rest, (*b*) during reactive hyperaemia.

The apparatus was calibrated before and after each examination in the following manner. Ten ml of water 37°C were injected into the plethysmograph and removed by suction, during which the deflection of the recorder pointer was noted and the calibration constant could be determined in mm/ml. By measuring the amount of water in the plethysmograph at the end of the examination, the volume of the calf segment could be calculated. For further details about the apparatus see Dahn (6).

Procedure. The examinations were performed in the afternoon (1–3 p.m.) with the patient in the supine position and with both legs placed in the plethysmograph (Fig. 1). In order to reach a state as basal as possible, 5 mg of diazepam was administered orally two hours before the examination. In the dose administered the drug is considered not to effect the circulation (22). The lower legs were positioned at a level of midthoracic height, i.e. approximately at the level of the right auricle. The room was quiet and the ambient temperature 22°C.

After the patient had lain with her lower legs enclosed in the plethysmographs for approximately 20 min, the arterial occlusion cuffs were applied at a pressure of 250 mmHg around both ankles. About one minute later venous occlusion cuffs were applied to each thigh just above the knee joints and the recordings were made. An occluding pressure of 55 mmHg was used.

Reactive hyperaemia was then induced by arterial occlusion in the thigh at a pressure of 250 mmHg for 5 min in combination with muscular exercise on the Thulesius foot ergometer (27) during the first 4 min.

The arterial occlusion cuff at the ankle was then re-applied and the occluding pressure on the thigh was released after reapplication. Recordings were made on the kymograph after applying the venous occlusion cuff to the thigh. In this manner recordings were made of the first flow of the peak flow—which, on the average, appeared 30 sec after releasing the arterial occlusion pressure—and of additional flows at intervals of approximately 15 sec during 3–5 min of the period of reactive hyperaemia. After this period the rates of flow had generally reached the magnitude prevailing at rest. Plethysmograms at rest and during reactive hyperaemia are illustrated in Fig. 2.

Calculations. The blood flow rates were calculated from the plethysmograms and the slope of the volume curve in relation to the time axis was determined. In case the increasing volume was not linear during the entire period of venous occlusion, the measurement of the slope was estimated during the first 3–4 pulse beats. The flow was calculated from the following formula. $F = (1\,000\ h)/(c\ V)$ where F is the flow in ml/min 100 ml tissue, h is the extrapolated perpendicular rise of the flow curve slope in 6 seconds, c is the calibration constant in mm/ml, and V is the volume of the leg segment in ml obtained by subtracting the drained volume of water from the volume of the plethysmograph (3 470 ml) at the end of the examination.

The error of a single measurement was calculated from six double determinations on two consecutive days and was found to be 19% of the mean in determination of the peak blood flow and 32% of the mean in determination of the resting blood flow. The error of measurement on the same day was calculated from twelve double determinations and was found to be 10% of the mean in determination of the resting blood flow. The calculations of the methodological error were performed according to Ehrhoff (8).

Determination of haemoglobin concentration

The haemoglobin concentration was determined in peripheral blood at the time of the plethysmographic examination. The determinations were performed spectrophotometrically at a wavelength of 544 nm (oxyhaemo-

Table II *Blood flow (ml/min × 100 ml tissue) in the lower legs at rest and at the peak flow during reactive hyperaemia*

Measurements during primipregnancy at different stages and after abortion or delivery Mean ± S.D RL = right leg; LL = left leg

	Resting blood flow			Peak blood flow		
	Pregnancy	After pregnancy	Difference	Pregnancy	After pregnancy	Difference
Group A	n = 27	n = 27		n = [illegible]7	n = 27	
RL	2.8±0.8	3.4±1.6	−0.5	30.4±9.0	35.6±11.7	−5.3
LL	3.1±1.0	3.1±1.3	0	31.2±7.8	31.4± 9.1	−0.2
Mean RL and LL	2.9±0.8	3.[illegible]±1.4	−0.3	30.8±7.7	33.5± 9.5	−2.7
Diff RL and LL	−0.2	+0.3		−0.9	+4.[illegible]	
Group B	n = 16	n = 15		n = 16	n = 15	
RL	2.7±1.0	[illegible]6±1.0	0	30.4±9.2	33.7±1[illegible].3	−3.9
LL	2.4±0.8	[illegible]5±0.7	−0.1	27.5±8.4	29.9± 8.9	−2.4
Mean RL and LL	2.6±0.8	2.5±0.8	0	29.0±8.3	31.9± 9.9	−3.1
Diff RL and LL	+0.2	+0.1		+2.9	+3.8	
Group C	n = 25	n = 20		n = 25	n = 19	
RL	2.0±0.7	2.0±0.9	0	29.0±9.7	[illegible]5.6±6.8	+3.9
LL	1.9±0.8	1.9±0.6	+0.1	24.5±7.3	[illegible]4.3±6.8	+1.2
Mean RL and LL	2.0±0.7	2.0±0.7	+0.1	26.7±7.8	[illegible]5.0±6.4	+2.5
Diff RL and LL	+0.1	+0.2		+4.5	+1.3	
Differences between groups (mean RL and LL)						
A–B	+0.3	+0.7		+1.8	+1.6	
A–C	+0.9	+1.2		+4.1	+8.5	
B–C	+0.6	+0.5		+[illegible]3	+6.9	

(0.01 < P ≤ 0.05). (0.001 < P < 0.01). (P < 0.001).

globin) after adding 0.04% ammonium hydroxide The error of a single measurement was calculated from twelve double determinations from fingertip blood and found to be 1.0

Statistical method

The mean value and standard deviation (S.D.) were calculated by conventional statistical methods (9). In the tables and figures the mean values and standard deviations are indicated. The differences were tested by the Student t test and the probability of the differences (P) was indicated at one of the following three levels: 0.01 < P ≤ 0.05 (* or almost significant), 0.001 < P < 0.01 (* or significant) and P ≤ 0.001 (* or highly significant)

RESULTS

As far as resting blood flow is concerned there were no significant differences between the blood flow in the right and the left lower leg in any of the three groups examined (Group A, Group B and Group C) The peak blood flow was somewhat higher in the right than in the left leg in Group A 2 weeks after abortion and in Group C during pregnancy For comparisons between the groups the individual mean value for both legs was used.

The resting blood flow was lower in the second half of pregnancy (Group C) than in the first half (Group A and Group B). During reactive hyperaemia no corresponding difference of peak blood flow seemed to exist between the second half of pregnancy and the first half (Table II).

The leg blood flow following abortion or delivery (Group B and Group C) was almost the same as the corresponding flow during pregnancy This is also true of resting blood flow in Group A. However during reactive hyperaemia the peak blood flow was lower in early pregnancy (Group A) than 2 weeks after early abortion

The blood flow during reactive hyperaemia was recorded for a period of 3 min and the values are shown on the graph in Fig 3 On an average the peak blood flow was reached 30 sec after releasing the arterial occlusion cuff As a rule one or two recordings had already been made before the peak value was reached

The haemoglobin concentration increased in

all groups after abortion or delivery but these changes have been disregarded. The small differences between the groups according to Table I are not statistically significant.

DISCUSSION

The average age of the women in Group C is slightly higher than in Groups A and B. The average age in all groups is relatively low and it does not seem likely that differences in blood flow in the three groups could be ascribed to differences in age (26).

The inconsistent differences in peak blood flow between right and left leg 2 weeks after abortion in Group A and during pregnancy in Group C are not clearly understood and are being further investigated. In the present study however these probable differences are of little interest.

The lower hyperaemic blood flow during early pregnancy compared with the corresponding blood flow after early abortion may be connected with a small increase in vascular resistance and a slight decrease in mean blood pressure in the legs (22).

As a substantial part of the case series was subjected to legal abortion it was possible to evaluate the parameters studied also in the nonpregnant state and to compare the pregnant and nonpregnant states. An investigation of five subjects revealed that the blood flow in the lower legs 2 weeks after early abortion was almost the same as that determined about 8 weeks following early abortion (mean difference 0.2 ± 1.2 ml/min 100 ml tissue for the rest flow and 1.4 ± 8.1 ml/min 100 ml tissue for the peak flow). Consequently the circulatory findings 2 weeks after early abortion were considered to be in good agreement with nonpregnant findings. Corresponding investigations were not performed in Groups B and C and in these groups the values post abortum and post partum might not be representative of the nonpregnant state (15, 16). Hence differences between the groups with respect to these later values may express a delayed restitution of peripheral blood flow after pregnancies of longer duration.

The lower resting blood flow in the legs at the end of pregnancy is not consistent with earlier findings of other investigators (1, 10, 15, 16), but supports the theory advanced by Hytten (18) which has not previously been confirmed ex-

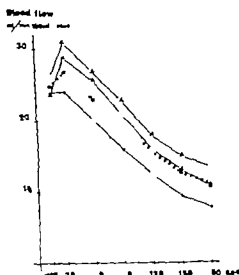


Fig. 3. Blood flow (ml/min 100 ml tissue) in reactive hyperaemia during primipregnancies of different lengths (Groups A, B and C) and fourteen days after abortion in Group A. ▲—▲ Group A in pregnancy; △—△ group A after abortion; ●—● group B in pregnancy; +—+ group C in pregnancy.

perimentally. According to this theory alterations in the blood flow through the uterus and placenta during the growth of these organs and the fetus and compression of the pregnant uterus on the large veins and arteries may in the supine position give rise to a lower resting blood flow distal to the uterus, i.e. in the legs. Increase in the blood flow through the arm at rest during the course of pregnancy (24) gives some credence to this line of reasoning. Although the case series of Spetz (24) consists of both primi- and multigravidas, this fact may not influence comparison with the present study. Blood flow changes caused by previous pregnancy are not as likely to take place in the arm as in the leg, where, among other things, mechanical effects occur.

The plethysmographically measured blood flow in the lower leg is mainly an expression of the muscular flow and therefore the present study cannot rule out changes in the skin blood flow. In the vasoregulatory asthenia syndrome (VA) the resting muscular blood flow however seems to be increased (4, 12). If in early pregnancy a hyperkinetic circulation is present in the legs, this should mainly affect nonmuscular tissue. Increased blood flow through the kidneys, and pos-

sibly through the uterus and the skin may thus mainly explain the increased cardiac output observed during early pregnancy (18).

REFERENCES

1 Abramson, D I, Flachs, K. & Fierst, S. M Amer J Obstet Gynec *45* 666, 1943
2. Bollinger A. Durchblutungsmessungen in der klinischen Angiologie Verlag Hans Huber Bern und Stuttgart, 1969
3 Burt, C. C. Edinb Med J Trans Edinb Obstet Soc *57* 18, 1950.
4 Carlsson, L., Pernow B. & Zetterquist, S. Acta Med Scand *17* 389 1962.
5 Dahn, I: Scand J Clin Lab Invest *16* 347 1964
6 — Acta Chir Scand *130* 4., 1965
7 — On the diagnosis of obliterative arterial disease with special reference to venous occlusion plethysmography Berlingska Boktryckeriet Lund, 1965
8. Enghoff, H. Uppsala universitets Arsskrift *9* 108, 1937
9 Freund, J E. Modern Elementary Statistics. Prentice Hall, Inc., Englewood Cliffs, New Jersey 1967
10. Ginsburg, J & Duncan, L. B. Sheila Cardiovasc Res *1* 13., 1967
11 Goodrich, S. M & Wood, J E: Amer J Obstet Gynec *90* 740, 1964
12. Graf, K. & Ström, G Arch Kreislaufforsch *50* 31 1966.
13. Greenfield, A. D M Methods in medial research. Year Book Chicago *8* 302, 1960.
14 Greenfield, A. D. M Whitney R. J & Mowbray J F Brit Med Bull *19* 101 1963.
15 Herbert, C. M Banner E. A. & Wakim, K. G. Amer J Obstet Gynec *68*, 1553 1954
16. — Amer J Obstet Gynec *76*, 742, 1958.
17 Holmberg, N G. & Sandström, B. Lecture at Congress of the Swedish Med. Assoc., Stockholm, 1970. Acta Obstet Gynec Scand *51* 143 1972.
18. Hytten, F E. & Leitch, Isabella. The Physiology of Human Pregnancy Blackwell Scientific Publications, Oxford, 1964
19 Kappert, A., Praxis *55* 806, 1966.
20. MacGregor W G & Snodgrass, C. A. Int J Gynaec Obstet *8*, 257 1970.
21 Sandström, B., Acta Obstet Gynec Scand *50* 71, 1971
— Sandström, B. & v Schmalensee G., Acta Obstet Gynec Scand 1972 *b* In preprint.
23 Shepherd, J T Physiology of the circulation in human limbs in health and disease Saunders, Philadelphia, 1963
24 Spetz, S., Acta Obstet Gynec Scand *43* 309 1964
25 Spetz, S. & Jansson, L., Acta Obstet Gynec Scand *48* 285 1969
26. Steinle H Plethysmographische Messung der Extremitäten—Durchblutung bei kreislaufgesunden Versuchspersonen. Inauguraldissertation, Tübingen, 1965.
27 Thulesius, O Scand J Clin Lab Invest *15* 550, 1963.

Submitted for publication Feb 10 197

Bo Sandström
Department of Obstetrics and Gynaecology
University of Umeå
S-901 85 Umeå
Sweden

Acta Obstet Gynec Scand 52, 205–210, 1973

FERTILITY, MOTILITY AND PENETRATION IN CERVICAL MUCUS OF FREEZE-PRESERVED HUMAN SPERMATOZOA

Magnar Ulstein

From the Department of Obstetrics and Gynecology, Östra sjukhuset (Head: Professor Per Bergman), Göteborg, Sweden

Abstract. Fertility, pre-freeze and post-thaw sperm motility, penetration ability and duration of motility in cervical mucus of spermatozoa were studied in 7 heterologous insemination donors and in 4 men in connection with homologous inseminations. No conception occurred after 50 heterologous and 15 homologous inseminations using semen preserved at −196°C. The post-thaw motility, penetration ability and duration of motility in cervical mucus of spermatozoa were reduced by the freeze-preservation procedure. The fertilizing capacity of freeze-preserved semen was more reduced than expected from the post-thaw sperm motility and penetration ability. The semen quality was of significant importance for the post-thaw sperm motility and penetration ability.

The fact that spermatozoa survive freezing and thawing has been known for almost 200 years (9, 17). Freezing of human semen was first tried by Jahnel (7). In 1949 the advantages of using glycerol as protective medium was described by Polge, Smith & Parkes (13). Bunge & Sherman (4) reported in 1953 the results of experiments with glycerol-treated human semen stored at −70 C. Since then numerous reports on inseminations with freeze-preserved human semen have appeared in the literature (2, 3, 5–10, 12, 14). Several methods for freezing and thawing have been tried (16). With all procedures there is a decrease in sperm motility and fertilizing capacity, but the post-thaw motility has poor correlation with fertility (2).

Preservation of semen by freezing has practical advantages. The semen can be received and frozen at a time convenient to donors and laboratory. There is more option in the choice of donors. The ejaculates can be divided into several portions, permitting the patients to be inseminated several times from the same donor in each cycle. For homologous inseminations several ejaculates can be collected for insemination at the time of ovulation.

The aim of the present investigation was to study the influence of freezing and thawing of semen on motility, penetration ability in cervical mucus, and fertilizing capacity of spermatozoa.

MATERIAL

A total of 65 ejaculates was frozen and thawed; 50 specimens came from 7 heterologous insemination donors, and 15 from 4 men with whom homologous inseminations were attempted.

The women who were inseminated belonged to the sterility clientele of the clinic and were apparently fertile. Fifty heterologous inseminations were performed in 14 women, and 15 homologous inseminations were performed in 4 women.

The women who did not conceive with freeze-preserved semen were inseminated with fresh semen. With this technic also 50 heterologous and 15 homologous inseminations were performed. The same donors were used.

METHODS

The fresh ejaculates, never more than half an hour after ejaculation, were delivered to the laboratory and preparation for freezing was immediately started.

Semen analyses were performed according to the routine of the laboratory. The volume of the ejaculate was measured. *Sperm density* was counted in a Bürker chamber. The percentage of motile spermatozoa and motility degree were determined by microscopy in a drop of well mixed semen. The proportion of motile spermatozoa was estimated to the nearest 5%. The degree of motility was registered numerically from 0 to 4. The spermatozoal morphology was studied and the concentration of fructose and acid phosphatases in seminal plasma were assessed.

Test of sperm penetration in cervical mucus was performed with a capillary tube technic described by Kremer

sibly through the uterus and the skin may thus mainly explain the increased cardiac output observed during early pregnancy (18).

REFERENCES

1 Abramson, D. L, Flachs, K. & Fierst, S. M., Amer J Obstet Gynec *45* 666, 1943
2. Bollinger A. Durchblutungsmessungen in der klinischen Angiologie. Verlag Hans Huber Bern und Stuttgart, 1969
3. Burt, C. C. Edinb Med J Trans Edinb Obstet Soc *57* 18, 1950.
4 Carlsson, L., Pernow B. & Zetterquist, S., Acta Med Scand *172* 389 1962.
5 Dahn, L. Scand J Clin Lab Invest *16*. 347 1964
6. — Acta Chir Scand *130* 4., 1965
7 — On the diagnosis of obliterative arterial disease with special reference to venous occlusion plethysmography Berlingska Boktryckeriet, Lund, 1965
8. Engholl, H. Uppsala universitets Arsskrift 9 108 1937
9 Freund, J E. Modern Elementary Statistics. Prentice Hall, Inc., Englewood Cliffs, New Jersey 1967
10. Ginsburg, J & Duncan, L. B. Sheila. Cardiovasc Res *1* 132, 1967
11 Goodrich, S M. & Wood, J E.: Amer J Obstet Gynec *90* 740, 1964.
12. Graf, K. & Ström, G., Arch Kreislaufforsch *50* 231 1966.
13 Greenfield, A. D M., Methods in medial research. Year Book, Chicago *8* 302, 1960.
14 Greenfield, A. D M., Whitney R. J & Mowbray J F Brit Med Bull *19* 101 1963
15 Herbert, C. M Banner E. A. & Wakim, K. G Amer J Obstet Gynec 68. 1553 1954
16. — Amer J Obstet Gynec *76* 742, 1958.
17 Holmberg, N G. & Sandström, B., Lecture at Congress of the Swedish Med. Assoc., Stockholm, 1970. Acta Obstet Gynec Scand *51* 143, 1972.
18 Hytten, F E. & Leitch, Isabella: The Physiology of Human Pregnancy Blackwell Scientific Publications, Oxford, 1964.
19 Kappert, A. Praxis *55* 806, 1966.
20. MacGregor W G. & Snodgrass, C. A., Int J Gynaec Obstet *8* 257 1970.
21 Sandström, B., Acta Obstet Gynec Scand *50*:71 1971
22. Sandström, B. & v Schmalensee G., Acta Obstet Gynec Scand *1972 b* In preprint.
23 Shepherd, J T Physiology of the circulation in human limbs in health and disease Saunders, Philadelphia, 1963.
24 Spetz, S., Acta Obstet Gynec Scand *43* 309 1964
25 Spetz, S. & Jansson, L. Acta Obstet Gynec Scand *48*. 285 1969
26. Steinler H Plethysmographische Messung der Extremitäten—Durchblutung bei kreislaufgesunden Versuchspersonen. Inauguraldissertation, Tübingen, 1965.
27 Thulesius, O Scand J Clin Lab Invest *15*. 350, 1963.

Submitted for publication Febr 10, 1972

Bo Sandström
Department of Obstetrics and Gynaecology
University of Umeå
S-901 85 Umeå
Sweden

Acta Obstet Gynec Scand 52: 205–210, 1973

FERTILITY MOTILITY AND PENETRATION IN CERVICAL MUCUS OF FREEZE PRESERVED HUMAN SPERMATOZOA

Magnar Ulstein

From the Department of Obstetrics and Gynecology Östra sjukhuset (Head, Professor Per Bergman), Göteborg, Sweden

Abstract. Fertility pre-freeze and post-thaw sperm motility penetration ability and duration of motility in cervical mucus of spermatozoa were studied in 7 heterologous insemination donors and in 4 men in connection with homologous inseminations. No conception occurred after 50 heterologous and 15 homologous inseminations using semen preserved at −196°C. The post-thaw motility penetration ability and duration of motility in cervical mucus of spermatozoa were reduced by the freeze-preservation procedure. The fertilizing capacity of freeze-preserved semen was more reduced than expected from the post-thaw sperm motility and penetration ability. The semen quality was of significant importance for the post-thaw sperm motility and penetration ability.

The fact that spermatozoa survive freezing and thawing has been known for almost 200 years (9 17). Freezing of human semen was first tried by Jahnel (7). In 1949 the advantages of using glycerol as protective medium was described by Polge, Smith & Parkes (13). Bunge & Sherman (4) reported in 1953 the results of experiments with glycerol-treated human semen stored at −70°C. Since then numerous reports on inseminations with freeze-preserved human semen have appeared in the literature (2, 3 5 10 12, 14). Several methods for freezing and thawing have been tried (16). With all procedures there is a decrease in sperm motility and fertilizing capacity but the post-thaw motility has poor correlation with fertility (7).

Preservation of semen by freezing has practical advantages. The semen can be received and frozen at time convenient to donors and laboratory. There is more option in the choice of donors. The ejaculates can be divided into several portions, permitting the patients to be inseminated several times from the same donor in each cycle. For homologous inseminations several ejaculates can be collected for insemination at the time of ovulation.

The aim of the present investigation was to study the influence of freezing and thawing of semen on motility penetration ability in cervical mucus, and fertilizing capacity of spermatozoa.

MATERIAL

A total of 65 ejaculates was frozen and thawed; 50 specimens came from 7 heterologous insemination donors, and 15 from 4 men with whom homologous inseminations were attempted.

The women who were inseminated belonged to the sterility clientele of the clinic and were apparently fertile. Fifty heterologous inseminations were performed in 14 women, and 15 homologous inseminations were performed in 4 women.

The women who did not conceive ith freeze-preserved semen were inseminated with fresh semen. With this technique also 50 heterologous and 15 homologous inseminations were performed. The same donors were used.

METHODS

The fresh ejaculates, never more than half an hour after ejaculation, are delivered to the laboratory and preparation for freezing was immediately started.

Semen analyses were performed according to the routine of the laboratory. The volume of the ejaculate was measured. Sperm density as counted in Buerker chamber. The percentage of motile spermatozoa and motility degree were determined by microscopy in drop of well mixed semen. The proportion of motile spermatozoa was estimated to the nearest 5%. The degree of motility was registered numerically from 0 to 4. The spermatozoal morphology was studied and the concentration of fructose and acid phosphatases in seminal plasma were assayed.

Test of sperm penetration in cervical mucus was performed with capillary tube technic described by Kremer

Table I *Semen properties pre freeze and post thaw motility of spermatozoa and sperm penetration in cervical mucus given as mean values for the ejaculates from each of the 11 donors*

Ranges in brackets

				Pre-freeze			Post-thaw		
Donor	Volume (ml)	Density (mill./ml)	Abnormal forms (%)	Motile (%)	Motility degree	Penetr (mm/3 h)	Motile (%)	Motility degree	Penetr (mm/3 h)
A	3.3 (3 0–3.5)	134 (105–150)	34 (31–37)	52 (55–70)	4	56 (53–57)	43 (40–45)	3	51 (48–55)
B	3 1 (2.5–3.5)	164 (150– 10)	29 (26–34)	55 (45–60)	4	34 (31 37)	45 (40–50)	3	28 (26–31)
C	2.4 (1.8–.6)	143 (110–174)	37 (32 39)	55 (45–65)	4	45 (42–48)	31 (25–40)	3	16 (1 18)
D	4.2 (2.2 5.3)	137 (100–152)	41 (37–43)	55 (50–60)	3	47 (42 51)	35 (30–40)		4 (19–7)
E	2.4 (2.0–2.6)	112 (105–150)	35 (3. 37)	54 (50–60)	3	36 (32 39)	27 (25–35)	2	1 (8–14)
F	2.5 (2.0–2.8)	131 (75–152)	48 (45–49)	48 (45–55)	3	49 (45–52)	29 (25–35)		9 (6–11)
G	2.9 (2.2 2.4)	80 (45–100)	47 (42 54)	44 (25–55)	3	27 (21–33)	17 (15–25)	.	9 (4–11)
H	2.6 (2.3–3.1)	120 (105–142)	62 (59–66)	50 (40–60)	3	21 (19–24)	.5 (15–35)		20 (18–.5)
I	6.5 (4 1 7.2)	25 (21 32)	61 (52 71)	30 (25–35)	2	5 (3–6)	5 (2 15)	1	0 (0–0)
J	3 1 (2.8–3.5)	55 (4 71)	63 (52–66)	35 (30–40)	2	11 (7–12)	15 (5–20)	2	3 (0–5)
K	3.8 (3.2 5.1)	71 (60–78)	43 (41–47)	40 (30–50)	2	15 (11 17)	5 (0–10)	1	0 (0–0)

(8) with slight modifications (18). Cervical mucus of ovulatory character carefully pretested, was used as test medium. The distance which the foremost spermatozoa had penetrated into the cervical mucus column in 3 hours was taken as the measure of linear penetration.

Duration of sperm motility in cervical mucus was tested in the same tubes as were used for the penetration test. After reading the penetration, incubation at 37°C was continued and at certain intervals the percentage of motile spermatozoa was estimated.

Freezing and thawing procedure The protective medium had the following composition. egg yolk, 20 by volume glycerol, 15 . by volume a solution of 5% glucose in water 26% by volume and a solution of 3 sodium citrate in water 39% by volume Glycine and erythromycin were added to this solution to concentrations of 15 mg and 1 mg per ml respectively This medium was heated to 56°C for 30 min and pH adjusted to 7.2 7.4 with 1.4 sodium bicarbonate solution. The medium was added to the ejaculates in the proportion 1 1 and an equilibration time of 15 min was allowed. The mixture of semen and protective medium was drawn into plastic straws with a length of 13 cm and a volume of about 0.5 ml. The ends of the straws were sealed with a moisture solidifying powder Slow cooling from 22°C to 4°C took place within 20 min. The plastic straws were then transferred to a rack and rapidly lowered into a container of liquid nitrogen in a manner which permitted the straws to be horizontally 4 cm above the liquid nitrogen surface where they were kept for 7 min. Then the straws were moved directly down into the liquid nitrogen, and stored at −196°C until thawed. Storage time varied from 1 day to 5 weeks.

The thawing was performed in a water bath at 37°C for 5 min.

Inseminations were performed as cervical and cap applications. Half a milliliter of the specimen was deposited in the cervical canal and 3–4 ml in a plastic cap on the portio. The ovulation time was estimated by basal body temperature length of cycle the cervical status, and the character of the cervical mucus. For heterologous insemination only one and for homologous insemination or 3 inseminations were performed each cycle

Statistical calculations Comparison of means was done with Student's t-test and significance established 5 level

RESULTS

For all semen samples the post-thaw spermatozoa showed decreased motility compared with fresh

Table II. *Grouping of the semen samples according to seminal properties*

The calculated mean penetration and mean motility recovery rates are given for each group.
S.E.M. standard error of the mean. n = number of samples

Semen property		Mean motility recovery rate	S.E.M.	Difference between the means	Mean penetration recovery rate	S.E.M.	Difference between the means
Density (mill./ml)							
60	16	33.3	5.3	17.0 ± 9.9	19.3	3.4	28.9 ± 12.4
> 60	49	50.3	2.4		48.2	3.1	
Motile (%)							
50	21	36.9	4.1	13.9 ± 9.6	28.2	4.3	19.7 ± 12.5
> 50	44	50.8	2.8		47.9	3.4	
Motility degree							
1 or 2	12	26.5	4.2	24.3 ± 3.9	14.2	3.1	33.5 ± 4.9
3 or 4	53	50.8	2.2		47.7	3.2	
Abnormal forms (%)							
> 50	11	33.0	3.7	15.7 ± 12.4	15.7	2.9	30.4 ± 15.0*
50	54	48.7	2.5		46.1	3.3	
Fructose (mg %)							
100	12	40.6	3.9	6.9 ± 5.9*	40.8	5.7	0.8 ± 4.5
100	53	47.5	2.7		41.6	3.5	
Acid phosphatase (IE)							
20 000	22	39.6	6.2	8.7 ± 17.6	40.6	3.7	1.8 ± 13.3
> 20 000	43	49.3	2.7		42.4	3.1	

* Significant

semen. The post-thaw sperm penetration of cervical mucus was for most of the semen samples decreased as compared with the pre-freeze values. However spermatozoa in some of the samples had an unchanged penetration ability. Table I shows the pre-freeze and post-thaw sperm motility and penetration in cervical mucus. The sperm motility and penetration ability are greatly reduced during the freezing and thawing of the semen samples of poor quality. For semen samples of good quality a reduction of 15–20 % motile spermatozoa was noted. The sperm penetration of the post-thaw samples varied from a complete loss of penetration ability to unchanged values, as compared with the pre-freeze specimens. The ranges of the percentage of motile spermatozoa and sperm penetration for different ejaculates from the same donor were small, both for the fresh and for the post-thaw specimens.

Motility degree of the spermatozoa in the post-thaw specimens was mostly one degree lower as compared with the fresh semen.

The motility recovery rate was calculated according to the equation: motility recovery rate = (% motile spermatozoa after thawing × 100)/% motile spermatozoa before freezing.

A similar equation was made for the penetration recovery rate: penetration recovery rate = (penetration extent after thawing × 100)/penetration extent before freezing.

The motility recovery rate varied from 11 to 89 and the penetration recovery rate varied from 0 to 100. Ejaculates from the same man had small ranges of motility and penetration recovery rate.

In Table II the semen samples are grouped according to seminal properties. For each group the mean penetration and motility recovery rates are calculated. The differences between means and their significance are indicated.

For semen samples with a density < 60 million/ml the mean motility and penetration recovery rates were significantly less than for samples with a density > 60 million/ml.

Semen samples having a percentage of motile spermatozoa ≥ 50 had a mean motility recovery rate and mean penetration recovery rate significantly greater than for samples having < 50 % of motile spermatozoa.

Semen samples with motility degree 3 or 4 had mean motility and penetration recovery rates significantly greater than semen samples with sperm motility degree 1 or 2.

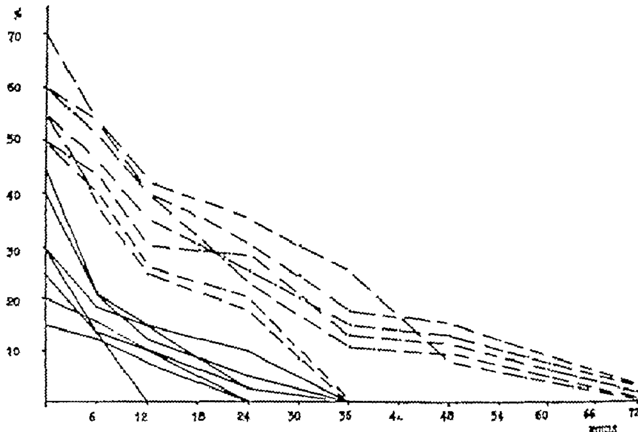


Fig. 1. Percentage of motile spermatozoa and duration of motility before and after freezing and thawing. Results with one ejaculate from each donor tested in cervical mucus. — — fresh semen —— after freezing and thawing.

The semen samples with percentage of abnormal spermatozoa ⩾ 50 had significantly lower motility and penetration recovery rates than semen samples with < 50 % abnormal spermatozoa.

For semen samples with ⩾ 100 mg % fructose in seminal plasma the mean motility recovery rate in contrast to the mean penetration recovery rate, was significantly greater than for samples with < 100 mg % fructose in seminal plasma.

For semen samples with ⩾ 20 000 IE/ml acid phosphatase in seminal plasma the mean motility recovery rate and penetration recovery rate were not significantly different from the means of samples with < 20 000 IE/ml acid phosphatase in seminal plasma.

The duration of sperm motility in the pre-freeze and post-thaw specimens is shown in Fig. 1. For fresh semen specimens the duration of sperm motility varied from 36 to 72 hours. The post-thaw specimens had a duration of motility from 12 to 36 hours. Already 6 hours after thawing a large reduction of motility had taken place.

Fifty heterologous inseminations in 14 women using freeze-preserved semen gave no conception. When using fresh semen from the same donors to the same women 8 conceptions occurred.

Fifteen homologous inseminations in 4 women using freeze-preserved semen gave no conception, but when using fresh semen one conception occurred.

DISCUSSION

The most serious difficulty in the use of freeze-preserved semen for inseminations is the reduced fertility and the inability to predict the fertility of the samples. Behrman & Sawada (2) stated that there was poor correlation between the motility recovery and the ability of the thawed semen to fertilize. Neither can predictions of the post thaw fertility be made from the fertility of a donor's freshly ejaculated semen (6).

In the present series no conceptions occurred using freeze preserved semen. When fresh semen from the same donors was used for heterologous inseminations 8 conceptions occurred. Thus it is obvious that freeze preserved semen has a greatly decreased fertilizing

fresh semen.

In a previous paper (19) it was shown that semen samples from the male partners of infertile marriages had a mean percentage of motile spermatozoa of 41.3. Most of the post-thaw specimens in the present work had a percentage of motile spermatozoa below that value, indicating reduced fertility. Matheson et al. (10) had successful inseminations with freeze-preserved semen samples where the post-thaw percentage of motile spermatozoa was 25–45. Behrman & Sawada (2) found best results for a donor where the post-thaw specimens had 25–30% motile spermatozoa. From these reports it can be concluded that the post-thaw motility is a poor predictor of fertility.

Furthermore, it has been shown that a degree of sperm motility of less than 3 indicates low fertility (19) and in the present material only a few post-thaw specimens had sperm motility of 3rd degree; the motility in the majority of specimens was below this value.

The post-thaw sperm penetration of cervical mucus for many of the semen samples was reduced as compared with the fresh samples, indicating reduction of fertility. In previous papers (19) it was shown that a penetration extent of 5 mm or less in 3 hours is associated with infertility. Subfertile semen samples had penetrations in the range of 6–19 mm in 3 hours. Semen from fertile men had penetrations of 20 mm or more in 3 hours. In the post-thaw specimens of the present work the penetrations were in the range of subfertility or infertility except for 3 of the donors.

The duration of sperm motility in cervical mucus of the post-thaw specimens was clearly reduced as compared with the fresh semen samples. For fresh donor semen there is a significant correlation between conception rate and duration of sperm motility (20), and the short duration of sperm motility of post-thaw specimens may give an explanation of the reduced fertility.

In the present investigation the post-thaw sperm motility, penetration ability and duration of sperm motility in cervical mucus corresponded well. Ackerman & Fjällbrant (6) also found that the post-thaw motility was the only factor which significantly predicted the penetration. Unfortunately a comparison of motility and penetration as indices of fertility is impossible with the present material. Matheson et al. (10) found that successful inseminations with freeze-preserved human semen occurred with samples where the penetration was good although the percentage of motile spermatozoa was low.

Sawada & Ackerman (14) found that spermatozoal motility recovery is related only to the initial motility and morphology, but in the present work the motility recovery rate was also significantly influenced by sperm density of the sample. It is therefore obvious that semen samples of poor quality are not suited for freeze-preserving.

The changes of sperm motility, penetration ability and duration of sperm motility which occur in connection with freezing and thawing of semen indicate a reduction in fertilizing potential. However, there seems to be a discrepancy between fertility and the results of the tests. Fertility is more reduced than would be expected. The spermatozoa are probably damaged during freezing and thawing more than is indicated by the motility and penetration ability. Ackerman (1, 15) has stated that the respiration of freeze-preserved spermatozoa is changed and Pedersen et al. (11) found structural changes of the acrosome region and the mitochondrial matrix, but how these changes influence the fertility is unknown.

From the present work it can be concluded that fertility of freeze-preserved semen is greatly reduced as compared with the fresh semen. The reduced fertility is partially explained by a reduction of motility, sperm penetration ability and duration of sperm motility in cervical mucus.

REFERENCES

1. Ackerman, D. R. Fructose utilization of human spermatozoa after cooling and freezing. Int J Fertil *12*: 1, 1967.
2. Behrman, S. J. & Sawada, Y. Heterologous and homologous inseminations with human semen stored in liquid nitrogen refrigerator. Fertil Steril *17*: 457, 1966.
3. Bunge, R. G., Keettel, W. C. & Sherman, J. K. Clinical use of frozen semen: A report of four cases. Fertil Steril *5*: 520, 1954.
4. Bunge, R. G. & Sherman, J. K. Fertilizing capacity of frozen human spermatozoa. Nature *172*: 767, 1953.
5. Fernandez-Cano, L., Menkin, M. F., Garcia, C. & Rock, J. Refrigerant preservation of human spermatozoa. I. Factors influencing recovery in euspermic semen. Fertil Steril *15*: 390, 1964.
6. Fjällbrant, B. & Ackerman, D. R. Cervical mucus penetration in vitro by fresh and frozen-preserved human semen specimens. J Reprod Fert *20*: 515, 1969.
7. Jahnel, F. Über die Widerstandsfähigkeit von menschlichen Spermatozoen gegenüber starker Kälte. Klin Wschr *17*: 1273, 1938.

8. Kremer J. The in vitro spermatozoal penetration test in fertility investigations. Dissertation, University of Groningen, The Netherlands, 1968.
9. Mantegazza, J Fisiologia sullo sperma umano. R.C. Ist. Lombardo 3 183 1866.
10. Matheson, G. W Carlborg, L. & Gemzell, C. Frozen human semen for artificial insemination. Amer J Obstet Gynec 104 495 1969
11 Pedersen, H. & Lebech, P Ultrastructural changes in human spermatozoa after freezing for artificial insemination. Fertil Steril 22 125 1971
12. Perloff, W H., Steinberger E. & Sherman, J K. Conception with human spermatozoa frozen by nitrogen vapor technique. Fertil Steril 15 501 1964
13 Polge G Smith, A. U & Parkes, A. S. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature 164 666 1949
14 Sawada, Y & Ackerman, D R. Use of frozen human semen. Progress in Infertility Little, Brown and Company Boston, 1968
15 Sawada, Y Ackerman, D R. & Behrman, S. J Motility and respiration of human spermatozoa after cooling to various low temperatures. Fertil Steril 18 775 1967
16. Sherman, J K. Improved methods of preservation of human spermatozoa by freezing and freeze-drying. Fertil Steril 14 46, 1963.
17 Spallanzani, L. Osservazioni, e Sperienze intorno ai Vermicelli, Spermatici dell'Uomo e degli Animali. Presso la Societa Tipographica, Modena, 1776.
18. Ulstein, M. Evaluation of the capillary tube sperm penetration method for fertility investigations. Acta Obstet Gynec Scand 51 287 1972.
19 Ulstein, M. Sperm penetration of cervical mucus as criterion of male fertility Acta Obstet Gynec Scand 51 335 1972.
20. Ulstein, M Fertility of husbands at homologous insemination. Acta Obstet Gynec Scand 5.. 5 1973.
1 Ulstein, M Fertility of donors at heterologous insemination. Acta Obstet Gynec Scand 5 97 1973

Submitted for publication March ?0 197?

Magnar Ulstein
Fylkessjukehuset i Alesund
6017 Aasestranda
Norway

Acta Obstet Gynec Scand 52: 211–220 1973

INVASIVE MOLE AND GESTATIONAL CHORIOCARCINOMA IN DENMARK, 1940–1969

Biological and Clinical Aspects

Bent Mogensen

From the Cancer Research Institute (Head Professor J. Bichel), the Danish Cancer Society Radiumstationen, Aarhus, Denmark

Abstract. A survey has been made of all invasive moles and gestational choriocarcinomata registered during a 30-year period in Denmark, a nation with a population of about 5 million (1971). All the diagnoses have been verified histologically by reappraisal. Based on clinical data the 2 groups of patients are described with reference to the following criteria: incidence and age; gravidity parity and latency; dissemination and course of disease; and treatment. Some case histories are reported which contrast with the view generally prevailing before the era of chemotherapy viz. that choriocarcinoma was a highly malignant and rapidly progressive disease. All the 19 patients who had an invasive mole, including 5 with metastases, became free from symptoms, and transition from this lesion to choriocarcinoma did not occur in any of these cases. Among the 50 choriocarcinoma patients the over-all mortality was 64%. 18 patients (36%) —10 of them with metastases—recovered. A decreasing mortality was observed during the period under investigation, but much additional work has to be done before the prognosis of the disease approaches that of invasive mole. In conclusion, the results of this study illustrate the many difficulties encountered in the diagnosis, treatment and follow-up of these rare trophoblastic tumours. The need for collaboration in prospective cases and for centralized treatment is strongly indicated from scientific as well as therapeutic points of view.

Recently a histological reappraisal was published on 94 cases originally diagnosed as choriocarcinoma or "probably choriocarcinoma" in Denmark during the period 1940–1969 (14). Based on the criteria laid down by the Armed Forces Institute of Pathology (6), 50 cases were classified as choriocarcinoma, 18 as invasive mole, and 26 as other trophoblastic proliferations. This survey includes the 68 trophoblastic tumours just mentioned and one additional case of invasive mole. It is reasonable to believe that these 69 patients represent all cases of invasive trophoblastic tumours, derived from fetal tissues, which occurred in Denmark in this period.[1]

Although invasive mole and choriocarcinoma are very rare neoplasms in Caucasians, neither the diagnosis nor the treatment of these diseases have been centralized in this country so far. Therefore, many pathologists, and even more clinicians, have been involved in the study and care of these patients who represent many admissions to many different hospitals. Below the two forms of tumours will be described separately. Formerly choriocarcinoma terminated fatally in 80–90% of the cases (17), in contrast to invasive mole which hardly ever runs a fatal course (10, 14, 20). After the introduction of chemotherapy with a folic-acid antagonist the prognosis of choriocarcinoma markedly improved (11), and today it is reasonable to believe that 80–90% of the patients will be cured under optimal conditions.

Since the foundation of the Danish Cancer Registry in 1942 all malignant neoplasms have been centrally registered (21), and this is the main reason why it has been possible to investigate the occurrence of these trophoblastic diseases in a whole nation during such a long period of time. Further it has been of importance that the histopathological service was limited to a few institutes before the organization of the registry. During the time under investigation the population of Denmark increased from 3.8 to 4.9 million, and

---

[1] Cases from the Faroe Islands and among the Eskimos in Greenland are not included. The populations in these communities are approx. 74 000 (1960).

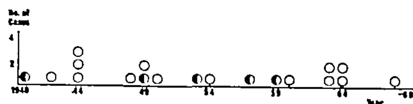


Fig 1 Nineteen cases of invasive mole related to the years of diagnosis and the spread of the disease. All the patients were cured of their disease. o, patients with localized disease; ◐ patients with deposits of tumour tissue beyond the myometrium.

the number of births per year averaged approx. 80 000.

Based on clinical data it is the purpose of this survey to present some biological characteristics of the 2 diseases, to report the outcome of their clinical management, and to emphasize the advantages of collaboration in prospective cases.

INVASIVE MOLE

Sometimes a significant invasion of the myometrium by one or more *molar villi* and associated trophoblast is observed. This condition is one manifestation of the lesion called invasive mole. The other manifestation is the production of (villous) metastases from a hydatidiform mole either with or without evidence of a primary lesion of the myometrium.

Incidence age

The 19 cases of invasive mole are related to the years of diagnosis and the spread of the disease in Fig. 1 The number of cases per year varied from 0 to 3 Localized disease was diagnosed in 14 patients and tumour tissue beyond the myometrium in 5

The age of the patients at the time of diagnosis varied from 17 to 54 years; 4 patients were more than 47 years old. The average age was 32.5 years for the whole group, and in the localized and generalized cases 35,2 and 25 0 years, respectively

Gravidity parity latency

Seven patients were primigravidae with a pregnancy resulting in a hydatidiform mole. In 12 multigravidae, the molar pregnancy was preceded by a total of 48 pregnancies, of which 39 resulted in full term deliveries and 9 in abortions. Thus, none of the patients had more than one hydatidiform mole. In this subgroup the ratio of deliveries to abortions, and moles was 3.3 0 7 1 0.

In 2 cases, the invasive mole was diagnosed simultaneously with a hydatidiform mole in the uterine cavity In the remaining 17 patients, the latent period between the diagnosis of the 2 types of moles varied from 5 days to 4 months, and averaged 1 6 months. Seven patients passed the hydatidiform mole or parts of it spontaneously and in 12 cases it was removed surgically

Dissemination and course of disease

At the time of diagnosis the invasive mole was limited to the myometrium in 14 patients, and during the course of disease no metastases were revealed in any of these cases. In 5 patients, deposits of tumour tissue were found beyond the myometrium at the time of diagnosis. Two patients had vaginal metastases and 2 tumour growth locally in the true pelvis. One of these 4 was the only case in which no primary tumour was revealed in the myometrium. Finally one patient had bilateral pulmonary metastases. Her primary tumour had penetrated the myometrium, and laparotomy disclosed large amounts of blood and clot" in the abdominal cavity A chest radiograph taken at the same time revealed a lung metastasis, and 3 more were demonstrated at later examinations. The metastases were not treated, and 5 months later they had disappeared. This is the only case in the present series presenting a spontaneous and lasting regression of metastases.

In this group, 4 out of 7 primigravidae had disseminated disease, as contrasted with one of the 12 multigravidae.

All the patients became free from symptoms; 18 are well today and one has died. Originally the latter patient was treated for the invasive mole by hysterectomy and excision of a vaginal metastasis plus external irradiation. Twenty four years later she presented with a poorly differentiated carcinoma of the bladder In order to exclude the possibility of recurrence of the previous trophoblastic disease measurements of human chorionic gonadotrophin (HCG) were performed twice Both investigations showed negative results. The cancer of the bladder metastasized, causing the death of the patient.

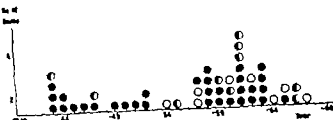


Fig. 2 Fifty cases of choriocarcinoma related to the years of diagnosis and the spread of the disease. ○, patients with localized disease. They are all well today. ◐ patients who recovered from generalized disease. ● patients who died of generalized choriocarcinoma, including one who died from postoperative complications (in 1946).

Treatment

All the 14 patients who had localized disease were subjected to hysterectomy with bilateral salpingo-oophorectomy in 8 cases and partial in 4. The surgical treatment was supplemented with external irradiation of the pelvis in 3 patients, and one had both external irradiation and an intravaginal application of radium. Finally additional cancer chemotherapy was given in 2 cases. One patient had a course of methylamethopterin (Methotrexate® total dose: 100 mg), and another was given cyclophosphamide (Endoxan® total dose: 2 400 mg) for a period of 3 months.

In one of the 5 patients who had tumour tissue beyond the myometrium the treatment was limited to excision of a paravaginal tumour plus external and internal irradiation. The other patients underwent hysterectomy and 2 were also subjected to bilateral and one to partial salpingo-oophorectomy. Furthermore, vaginal or pelvic tumour deposits were removed surgically in 3 patients, and additional external irradiation was given in 2.

CHORIOCARCINOMA

Following the rules which are now generally accepted, the criteria for the diagnosis of choriocarcinoma are briefly as follows: An invasively growing, haemorrhagic tumour consisting of malignant trophoblast without the occurrence of chorionic villi. Often, the tumour tissue has a plexiform mode of growth with bars of cytotrophoblast covered with festoons of syncytiotrophoblast. Both types of cells must always be present.

Incidence age

The 50 cases of choriocarcinoma are presented in relation to the years of diagnosis and the spread of the disease in Fig. 2. The number of patients per year differs considerably during the time under consideration. Seven cases were diagnosed in 1961, whereas no case was registered in 8 of the years. Further it is seen that 27 cases (54%) were registered in the 7-year period from 1957–63.

The ages of the patients ranged from 17 to 54 years at the time of diagnosis, and averaged 30.0 years. The average age of the 32 patients who died was 31.7 years as compared with 27.1 years among the 18 survivors. In the 10 patients who recovered after having disseminated disease the average age was 26.1 years.

Gravidity parity latency

In one case no preceding pregnancy had ever been noted before the diagnosis of choriocarcinoma. Eleven patients had only been pregnant once; their pregnancies resulted in 7 deliveries, 3 cases of hydatidiform mole and one abortion.

Among the 38 multigravidae, 125 previous pregnancies were recorded, which had resulted in 90 deliveries (72%), 17 abortions (14%) and 18

Table 1. *The pregnancy apparently antecedent to choriocarcinoma*

	Delivery	Hydatidiform mole	Abortion	Uncertain	Fatal	Non-fatal	Total
Primigravidae	7	3	1	1	8	4	12
Multigravidae	11	17	9	1	24	14	38
Total	18	20	10	2	32	18	50

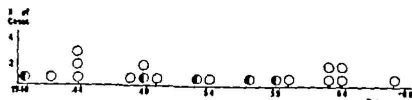


Fig 1 Nineteen cases of invasive mole related to the years of diagnosis and the spread of the disease. All the patients were cured of their disease. o, patients with localized disease; ◐ patients with deposits of tumour tissue beyond the myometrium.

the number of births per year averaged approx. 80 000.

Based on clinical data it is the purpose of this survey to present some biological characteristics of the 2 diseases, to report the outcome of their clinical management, and to emphasize the advantages of collaboration in prospective cases.

INVASIVE MOLE

Sometimes a significant invasion of the myometrium by one or more *molar villi* and associated trophoblast is observed. This condition is one manifestation of the lesion called invasive mole. The other manifestation is the production of *(villous) metastases* from a hydatidiform mole either with or without evidence of a primary lesion of the myometrium.

Incidence age

The 19 cases of invasive mole are related to the years of diagnosis and the spread of the disease in Fig. 1 The number of cases per year varied from 0 to 3 Localized disease was diagnosed in 14 patients and tumour tissue beyond the myometrium in 5

The age of the patients at the time of diagnosis varied from 17 to 54 years; 4 patients were more than 47 years old. The average age was 32.5 years for the whole group and in the localized and generalized cases 35,2 and 25 0 years, respectively

Gravidity parity latency

Seven patients were primigravidae with a pregnancy resulting in a hydatidiform mole. In 12 multigravidae, the molar pregnancy was preceded by a total of 48 pregnancies, of which 39 resulted in full term deliveries and 9 in abortions. Thus, none of the patients had more than one hydatidiform mole. In this subgroup the ratio of deliveries to abortions, and moles was 3 3 0, 7 1 0

In 2 cases, the invasive mole was diagnosed simultaneously with a hydatidiform mole in the uterine cavity In the remaining 17 patients, the latent period between the diagnosis of the 2 types of moles varied from 5 days to 4 months, and averaged 1 6 months. Seven patients passed the hydatidiform mole or parts of it spontaneously and in 12 cases it was removed surgically

Dissemination and course of disease

At the time of diagnosis the invasive mole was limited to the myometrium in 14 patients, and during the course of disease no metastases were revealed in any of these cases. In 5 patients, deposits of tumour tissue were found beyond the myometrium at the time of diagnosis. Two patients had vaginal metastases and 2 tumour growth locally in the true pelvis. One of these 4 was the only case in which no primary tumour was revealed in the myometrium. Finally one patient had bilateral pulmonary metastases. Her primary tumour had penetrated the myometrium, and laparotomy disclosed "large amounts of blood and clot" in the abdominal cavity A chest radiograph taken at the same time revealed a lung metastasis, and 3 more were demonstrated at later examinations. The metastases were not treated, and 5 months later they had disappeared. This is the only case in the present series presenting a spontaneous and lasting regression of metastases.

In this group 4 out of 7 primigravidae had disseminated disease, as contrasted with one of the 12 multigravidae.

All the patients became free from symptoms; 18 are well today and one has died. Originally the latter patient was treated for the invasive mole by hysterectomy and excision of a vaginal metastasis plus external irradiation. Twenty-four years later she presented with a poorly differentiated carcinoma of the bladder In order to exclude the possibility of recurrence of the previous trophoblastic disease measurements of human chorionic gonadotrophin (HCG) were performed twice. Both investigations showed negative results. The cancer of the bladder metastasized, causing the death of the patient.

ports), the same pattern is apparent in both groups. The shortest interval is observed after a delivery: it is somewhat longer after an abortion and longest after a mole. In the fatal group, the interval is longer after delivery and abortion and shorter after a mole as compared with the non-fatal cases. When all types of pregnancies are considered together the interval was almost identical in the 2 groups: 10.2 and 9.9 months. The survival time of the patients from diagnosis to death parallels the pattern of latency indicating that the disease progresses most slowly after a mole. The mean interval between the termination of the preceding pregnancy and death was 9.6 months after a delivery 12.8 months after an abortion, and 21.2 months after a mole.

The patients in whom choriocarcinoma developed after a delivery had the highest mortality. In this group, 13/18 (72%) died, and when the diagnosis was made after hydatidiform mole or abortion, the death rates were 12/20 (60%) and 6%/10 (60%), respectively.

Twenty-four patients had generalized disease at the time of diagnosis, and only 2 survived. Twenty-six patients had localized disease at the time of diagnosis; the disease remained localized in 9 of whom 8 survived and one died from postoperative complications. Metastases developed in 17 during the course of their disease. Nine of these patients died of their malignancy and 8 recovered.

Among the patients who died, 26 survived less than 8 months after diagnosis, 5 survived for 13–25 months, and one for almost 11 years. If the last-mentioned case is excluded, the survival time with choriocarcinoma averaged 5.0 months.

Case reports

It is well known that the symptomatology and the course of choriocarcinoma can vary within wide limits. Nevertheless, 4 exceptional cases are briefly reported below.

The first 2 patients are both examples of a long latent period, one patient died, the other survived.

Case 19. A woman, aged 38, gravida 1, para 1, had curettage to evacuate mole in March 1949. Ten months later she had an abortion, and after another 15 months (April 1951) acute endometritis was diagnosed. Seven years and 4 months after the abortion the patient was admitted to hospital with haemoptysis, and chest radiography revealed multiple metastases in both lungs. Histological examination of an isolated, subcutaneous metastasis proved the diagnosis of choriocarcinoma. The patient died of the disease 2 months later (June 1957).

Case 47. The patient, aged 24, gravida 2, para 1 passed mole in January 1961. Five years later (January 1966), the patient had recurrent bronchitis, and chest radiography showed tumour of the right lung. Pneumonectomy was performed, and histological examination of the tumour revealed diagnosis of choriocarcinoma. Two months after the operation the patient was subjected to hysterectomy but both gross and histological examinations failed to reveal tumour tissue in the myometrium. Today the patient is well, and she has now been free of symptoms for nearly 6 years.

The next patient illustrates a very protracted course of the disease with a fatal outcome.

Case 28. The patient, aged 31 gravida 2, para 2, had curettage to evacuate a mole in February 1959. Owing to irregular vaginal bleeding, the patient was subjected to hysterectomy one year later (March 1960), and a choriocarcinoma in the myometrium was demonstrated. Four months later (July 1960), chest radiography revealed 2 metastases in the right lung. The metastases persisted for 10 months, and then they disappeared completely without treatment. In January 1964, the patient was readmitted with diagnosis of pleurisy and now metastases were present in the left lung. From February 1964 to March 1966, the patient was admitted 20 times for cancer chemotherapy (Methotrexate and vinblastine (Velbe®)). Pneumonectomy was performed in August 1966, the histological diagnosis was one of metastases from choriocarcinoma. In 1968–69 the patient was immunized twice with skin transplants and twice with lymphocytes and platelets from her husband. During 1970 another 6 courses of Methotrexate were given. She died of her disease in January 1971, and autopsy revealed a tumour thrombus in the right pulmonary artery. Histological examination of the thrombus confirmed the diagnosis of choriocarcinoma. Apart from the thrombus, no other metastases were found. It is noteworthy that this patient survived with generalized choriocarcinoma for nearly 11 years, and she had no subjective feeling of being ill during the greater part of this period.

The last patient represents a case of latent choriocarcinoma (3) which was cured.

Case 30. The patient, aged 34, gravida 8, para 7 had mole evacuated in October 1947. Owing to profuse vaginal bleeding hysterectomy was performed one month later (November 1947), and choriocarcinoma was demonstrated in the myometrium. After this episode, the patient felt perfectly well, but 8 years later (December 1955) she was admitted to hospital with tumour of the right labium minus. The tumour was excised and the histopathological diagnosis was choriocarcinoma. Today the patient has been free from symptoms of the disease for more than 16 years.

Many more exceptional case histories could be mentioned, and it is reasonable to conclude that

Table II *Diagnosis of choriocarcinoma and survival with the tumour related to the type of antecedent pregnancy*

Type of antecedent pregnancy	Total	Fatal	Mean interval between: Termination of pregnancy and diagnosis (months)	Mean interval between: Diagnosis and death (months)	Non-fatal	Mean interval between termination of pregnancy and diagnosis (months)
Delivery	18	13	7.0	2.6	5	4.4
Hydatidiform mole	20	12	14.8	6.4[b]	8	15.3
Abortion	10	6	7.6[a]	5.2	4	6.3
Uncertain	2	1	—	0.6	1	—
Total	50	32	10.2 (30 pts)[a]	5.0[b]	18	9.9 (17 pts)

[a] Patient 19 is omitted. In this case, 88 months passed between pregnancy and diagnosis.
[b] Case 28 is excluded. This patient lived with choriocarcinoma for nearly 11 years.

cases (14%) of hydatidiform mole. The average number and the outcome of the pregnancies were almost identical in the 24 fatal and 14 non fatal cases in this group pregnancies 3.3/3.3 deliveries 2.4/2.4 abortions 0.45/0.4 and moles 0.45/0.5

The period of time from the termination of the last pregnancy to the diagnosis of choriocarcinoma was known in 48 of the 50 patients. The latent interval varied from one to 88 months, and averaged 11.7 months. Thirty-three cases were diagnosed within the first year after the termination of the antecedent pregnancy 10 within the second, and in 5 cases the latent period exceeded 2 years. If the last 5 cases are excluded the latency of the remaining 43 cases averaged 7.3 months.

The pregnancy apparently antecedent to choriocarcinoma

In 18 patients (36%) choriocarcinoma developed after a delivery (Table I). Twelve of the children born were girls and 6 were boys. In 17 of the cases, the infants were alive at birth and apparently healthy 16 of them were born at term and one 10 weeks before term. One child was stillborn (no autopsy) although the pregnancy had been perfectly normal Another child died 9 hours after birth, autopsy revealed minor haemorrhages in the cerebellar tentorium and in the cerebral falx. A third child (girl) died at the age of 18 months of a virilizing tumour of the adrenal cortex, which had been diagnosed 4 months previously At autopsy metastases from this tumour were found in several organs, but no signs of choriocarcinoma were demonstrated. Thus, two forms of cancer had developed from the gestation in question but the first tumour the choriocarcinoma, metastasized only to the mother who died with generalized disease 8 months after the delivery The remaining 15 children are all alive today and their survival is being regularly followed.

In 20 patients (40%) the pregnancy apparently antecedent to choriocarcinoma resulted in a hydatidiform mole and in 10 (20%) in abortion. At least one abortion was procured illegally and an other was a missed abortion in the 5th month. Finally the nature of the gestation preceding the diagnosis of choriocarcinoma could not be confidently assigned in 2 cases (4%). One of these patients was diagnosed as a case of a tubal choriocarcinoma. Eleven years previously her first pregnancy had resulted in a normal delivery and 8 years later she had an abortion in the 5th week. One year after this episode she began treatment for involuntary infertility and during this period she was inseminated 3 times apparently with negative result. One year after the last insemination irregular vaginal bleeding occurred which led to admission and diagnosis. In the second patient who was 54 years old, pregnancy had never been noted, and it cannot be excluded that this patient represents a case of choriocarcinoma ab initio (1).

Dissemination and course of disease

The mean interval between the termination of the apparently antecedent pregnancy and the diagnosis is related to the type of preceding pregnancy in both the fatal and non-fatal groups (Table II). If cases 19 and 28 are excluded (see Case Re

ports), the same pattern is apparent in both groups. The shortest interval is observed after a delivery. It is somewhat longer after an abortion and longest after a mole. In the fatal group, the interval is longer after delivery and abortion and shorter after a mole as compared with the non-fatal cases. When all types of pregnancies are considered together the interval was almost identical in the 2 groups: 10.2 and 9.9 months. The survival time of the patients from diagnosis to death parallels the pattern of latency indicating that the disease progresses most slowly after a mole. The mean interval between the termination of the preceding pregnancy and death was 9.6 months after a delivery 12.8 months after an abortion, and 21.2 months after a mole.

The patients in whom choriocarcinoma developed after a delivery had the highest mortality. In this group, 13/18 (72%) died, and when the diagnosis was made after hydatidiform mole or abortion, the death rates were 12/20 (60%) and 6%/10 (60%), respectively.

Twenty-four patients had generalized disease at the time of diagnosis, and only 2 survived. Twenty-six patients had localized disease at the time of diagnosis; the disease remained localized in 9 of whom 8 survived and one died from postoperative complications. Metastases developed in 17 during the course of their disease. Nine of these patients died of their malignancy and 8 recovered.

Among the patients who died, 26 survived less than 8 months after diagnosis, 5 survived for 13–25 months, and one for almost 11 years. If the last-mentioned case is excluded, the survival time with choriocarcinoma averaged 5.0 months.

Case reports

It is well known that the symptomatology and the course of choriocarcinoma can vary within wide limits. Nevertheless, 4 exceptional cases are briefly reported below.

The first patients are both examples of a long latent period, one patient died, the other survived.

Case 19. A woman, aged 38, gravida 1, para 1 had curettage to evacuate mole in March 1949. Ten months later she had an abortion, and after another 15 months (April 1951) acute endometritis was diagnosed. Seven years and 4 months after the abortion the patient was admitted to hospital with haemoptysis, and chest radiography revealed multiple metastases in both lungs. Histological examination of an excised, subcutaneous metastasis proved the diagnosis of choriocarcinoma. The patient died of the disease 2 months later (June 1957).

Case 47. The patient, aged 24 gravida 2, para 1 passed mole in January 1961. Five years later (January 1966), the patient had recurrent bronchitis, and chest radiography showed a tumour of the right lung. Pneumonectomy was performed, and histological examination of the tumour revealed diagnosis of choriocarcinoma. Two months after the operation the patient was subjected to hysterectomy but both gross and histological examinations failed to reveal tumour tissue in the myometrium. Today the patient is well, and she has now been free of symptoms for nearly 6 years.

The next patient illustrates a very protracted course of the disease with a fatal outcome.

Case 38. The patient, aged 31, gravida 2, para 2, had curettage to evacuate mole in February 1959. Owing to irregular vaginal bleeding, the patient was subjected to hysterectomy one year later (March 1960), and choriocarcinoma in the myometrium was demonstrated. Four months later (July 1960), chest radiography revealed 2 metastases in the right lung. The metastases persisted for 10 months, and then they disappeared completely without treatment. In January 1964 the patient was readmitted with a diagnosis of pleurisy and new metastases were present in the left lung. From February 1964 to March 1966, the patient was admitted 20 times for cancer chemotherapy (Methotrexate and vinblastine (Velbe®)). Pneumonectomy was performed in August 1966 the histological diagnosis was one of metastases from choriocarcinoma. In 1968–69 the patient was immunized twice with skin transplants and twice with lymphocytes and platelets from her husband. During 1970 another 6 courses of Methotrexate were given. She died of her disease in January 1971 and autopsy revealed tumour thrombus in the right pulmonary artery. Histological examination of the thrombus confirmed the diagnosis of choriocarcinoma. Apart from the thrombus, no other metastases were found. It is noteworthy that this patient survived with generalized choriocarcinoma for nearly 11 years, and she had no subjective feeling of being ill during the greater part of this period.

The last patient represents a case of latent choriocarcinoma (3) which was cured.

Case 49. The patient, aged 34 gravida 8, para 7 had mole evacuated in October 1947. Owing to profuse vaginal bleeding hysterectomy was performed one month later (November 1947), and choriocarcinoma was demonstrated in the myometrium. After this episode, the patient felt perfectly well, but 8 years later (December 1955) she was admitted to hospital with tumour of the right labium majus. The tumour was excised and the histopathological diagnosis was choriocarcinoma. Today the patient has been free from symptoms of the disease for more than 16 years.

Many more exceptional case histories could be mentioned, and it is reasonable to conclude that

Table III *Methods of treatment related to the spread of the disease at the time of diagnosis*

		Surgery				Irradiation		Chemotherapy	No	
	No. of cases	Exclusively	+ Irradiation	+ Chemotherapy	+ Irradiation + chemotherapy	Exclusively	+ Chemotherapy	Exclusively	treatment	Total
Localized disease	26	14 (9)	6 (2)	2 (1)	4 (4)	0	0	0	0	26 (16)
Generalized disease	24	6 (1)	5 (1)	3 (0)	3 (0)	1 (0)	1 (0)	0	5 (0)	4 (2)
Total	50	20 (10)	11 (3)	5 (1)	7 (4)	1 (0)	1 (0)	0	5 (0)	50 (18)

The figures in brackets indicate the number of survivors.

it is not an easily recognizable disease with a uniform course but a condition which is often unexpected and unpredictable.

Treatment

The treatment of the patients varied considerably during the period under investigation. This seems to be due mainly to 3 factors: (1) decentralized treatment, (2) the introduction of new methods of treatment, and (3) the inclusion in any one centre of cases of varying severity and extent. For limitation and clarity of presentation the comments on the treatment of the patients are given in broad outline only

The methods of treatment are related to the spread of the disease at the time of diagnosis (Table III). Twenty-six *localized* cases—of which 17 became generalized during the course of disease—were all treated by surgery In addition, irradiation or/and chemotherapy were given to 12 patients.

Twenty-five patients underwent hysterectomy and 15 partial or bilateral salpingo-oophorectomy One patient was also subjected to pneumonectomy External irradiation was given in 10 cases, and half of these also had intravaginal radium therapy Finally 6 patients received Methotrexate and one of these had Velbe, too.

Among 24 patients with *generalized* disease, 5 died of their disease less than 8 days after admission or diagnosis, and no adequate treatment was given in any of these cases. Seventeen had operations, and 11 received supplementary treatment in the form of irradiation or/and chemotherapy One patient underwent irradiation exclusively and another irradiation plus chemotherapy

Hysterectomy and partial or bilateral salpingo-oophorectomy were performed in 14 cases. Additional surgical treatment was given to 4 patients; pulmonary metastases were removed in ? in one case a vaginal metastasis and in another a pelvic tumour was removed. Two patients underwent lobectomy alone and in a third patient this operation was performed in addition to the removal of an abdominal tumour Ten patients were given external irradiation, which in 2 was supplemented by radium therapy Finally 7 patients received chemotherapy One had triethylenemelamine (TEM®) 2 received Endoxan, and 4 Methotrexate, which in one case was supplemented by 1 4-dimethanesulfonate (1 Threitol®).

It is noteworthy that no patient had cancer chemotherapy as the only treatment. Further it should be added that in the total series 13 patients received chemotherapy and 11 of these had surgery first. Two of those who received chemotherapy and irradiation had both treatments simultaneously

DISCUSSION

In the present series, all the 19 patients who had an invasive mole were cured. Similarly no deaths referable to this type of trophoblastic disease occurred among 22 cases reported from Norway and Sweden (10, ?0) or in a series of 100 cases treated for trophoblastic neoplasia in Britain ( ) These findings agree with the generally accepted assumption that death from invasive mole is rare and mainly due to sepsis or haemorrhage

It is reported that 1.5–2.5% of hydatidiform moles give rise to choriocarcinoma (7 10 20), and it might be expected that, because of its aggressive mode of growth, invasive mole would more often develop into choriocarcinoma. However no such case was recorded in any of the Scandinavian and British series. Among 96 consecutive patients suffering from choriocarcinoma Ober et al. found only one (non-Caucasian) in whom the disease had arisen from the trophoblast of an invasive mole (18). Based on these premises there is no evidence suggesting that invasive mole is more likely to be associated with subsequent choriocarcinoma than hydatidiform mole—at least in Caucasians.

In evaluating the forms of treatment used for invasive mole in the present series it should be borne in mind that all the patients survived. On the other hand, it is an open question whether some of the patients were treated more intensively than necessary to obtain a cure. Gross and histological examination did not reveal tumour tissue in any of the excised ovaries, and the supplementary irradiation and chemotherapy given to a total of 9 patients were instituted before the effect of the surgical treatment could be assessed by changes in the excretion of HCG In 18 cases, the original histopathological diagnoses were either "invasive mole with transition into choriocarcinoma" or choriocarcinoma" and this is presumably one of the reasons why some of the patients were so intensively treated.

It is still an unanswered question why invasive mole, even before the introduction of cancer chemotherapy had a good prognosis. Today it is a common belief that cure of invasively growing, metastasizing neoplasms depends on the presence of active defence mechanisms in the patient, and evidence is available suggesting that immunological reactions take part in these mechanisms (8 22) This assumption presupposes that the tumour contains antigens which are foreign to the patient. It is unknown whether invasive mole is, in general, more antigenic than the choriocarcinoma, but it is reasonable to stress that unlike the choriocarcinoma the mole contains both fibroblasts of villous stromal origin and endothelial cells from fetal vessels.

On the basis of histological study of 43 invasive moles, Park wrote: It was quite exceptional for lymphocytes to be less than abundant in immediate or close proximity to the hyperplastic trophoblast; this was seen particularly in relation to the interstitially placed villi" (19). On histological examination of hysterectomy specimens from 8 patients we made the same observation (15). None of the patients were treated before surgery and the morphology and the predominantly perivascular position of the round cells were identical to those of the cellular allograft reaction. It also supports the antigenicity hypothesis" that in our series 4 out of 7 primigravidae had generalized disease, as contrasted with one among 12 multigravidae. This difference in the spread of the disease in the two groups is statistically significant at the 5% level ($p = 0.038$ Fisher's exact test). This finding is compatible with the assumption that the multigravidae may have been sensitized by previous pregnancies, and therefore may have been able—more rapidly and effectively—to establish an immunological defence against the tumour tissue.

Out of a total of 50 *choriocarcinoma patients*, 27 (54%) were registered during the 7-year period from 1957–1963 However this excess cannot be explained by improved diagnostic possibilities as the histopathological services have been constantly developing in the period under investigation, and only 6 cases were registered during the next 6 years

In this study the average age of the 32 patients who died exceeded by 5 6 years that of the 10 patients who recovered after the disease had become disseminated. Ober et al. found a similar age distribution in a series of 96 patients (16). With reservations because of the small numbers of patients, there might be a tendency for the prognosis to be better in younger than in older patients. The oldest patient in this group who was cured of generalized disease was 34 years old at the onset of the disease, while all the 7 patients who were older died of their disease. Thus age appears to be of prognostic significance.

When choriocarcinoma occurs after a delivery or an abortion, the physician never suspects the disease until after the development of symptoms, which in most cases consist of irregular vaginal bleeding. In contrast, any hydatidiform mole must be considered a potential precancerous lesion indicating the need for frequent clinical and laboratory investigations. Therefore, early diagnosis might be anticipated in the presence of a

Table III. *Methods of treatment related to the spread of the disease at the time of diagnosis*

		Surgery				Irradiation		Chemo-therapy	No	
	No. of cases	Exclusively	+ Irradiation	+ Chemo-therapy	+ Irradiation + chemo-therapy	Exclusively	+ Chemo-therapy	Exclusively	treatment	Total
Localized disease	26	14 (9)	6 (2)	2 (1)	4 (4)	0	0	0	0	26 (16)
Generalized disease	24	6 (1)	5 (1)	3 (0)	3 (0)	1 (0)	1 (0)	0	5 (0)	4 (2)
Total	*50*	*20 (10)*	*11 (3)*	*5 (1)*	*7 (4)*	*1 (0)*	*1 (0)*	0	*5 (0)*	*50 (18)*

The figures in brackets indicate the number of survivors.

it is not an easily recognizable disease with a uniform course but a condition which is often unexpected and unpredictable.

Treatment

The treatment of the patients varied considerably during the period under investigation. This seems to be due mainly to 3 factors: (1) decentralized treatment, (2) the introduction of new methods of treatment, and (3) the inclusion in any one centre of cases of varying severity and extent. For limitation and clarity of presentation the comments on the treatment of the patients are given in broad outline only

The methods of treatment are related to the spread of the disease at the time of diagnosis (Table III). Twenty-six *localized* cases—of which 17 became generalized during the course of disease—were all treated by surgery In addition irradiation or/and chemotherapy were given to 12 patients.

Twenty-five patients underwent hysterectomy and 15 partial or bilateral salpingo-oophorectomy One patient was also subjected to pneumonectomy External irradiation was given in 10 cases, and half of these also had intravaginal radium therapy Finally 6 patients received Methotrexate and one of these had Velbe too.

Among 24 patients with *generalized* disease 5 died of their disease less than 8 days after admission or diagnosis, and no adequate treatment was given in any of these cases. Seventeen had operations, and 11 received supplementary treatment in the form of irradiation or/and chemotherapy One patient underwent irradiation exclusively and another irradiation plus chemotherapy

Hysterectomy and partial or bilateral salpingo-oophorectomy were performed in 14 cases. Additional surgical treatment was given to 4 patients; pulmonary metastases were removed in ? in one case a vaginal metastasis and in another a pelvic tumour was removed. Two patients underwent lobectomy alone, and in a third patient this operation was performed in addition to the removal of an abdominal tumour Ten patients were given external irradiation, which in 2 was supplemented by radium therapy Finally 7 patients received chemotherapy One had triethylenemelamine (TEM®) 2 received Endoxan, and 4 Methotrexate which in one case was supplemented by 1 4-dimethanesulfonate (1 Threitol®).

It is noteworthy that no patient had cancer chemotherapy as the only treatment. Further it should be added that in the total series 13 patients received chemotherapy and 11 of these had surgery first. Two of those who received chemotherapy and irradiation had both treatments simultaneously

DISCUSSION

In the present series, all the 19 patients who had an *invasive mole* were cured. Similarly no deaths referable to this type of trophoblastic disease occurred among 22 cases reported from Norway and Sweden (10, ?0) or in a series of 100 cases treated for trophoblastic neoplasia in Britain (?). These findings agree with the generally accepted assumption that death from invasive mole is rare and mainly due to sepsis or haemorrhage

be reasonable to end up with some current considerations about problems related to invasive mole and choriocarcinoma.

Intensive and prolonged observation of patients who have had a hydatidiform mole will allow a diagnosis of invasive mole and choriocarcinoma to be made earlier than seen in this study. Many more difficulties are involved in the early diagnosis of choriocarcinoma following abortion or delivery. The occurrence of irregular and profuse vaginal bleeding should arouse suspicion of a primary tumour provided that the result of curettage is inconclusive. However some choriocarcinomata develop deep in the myometrium without causing any bleeding into the uterine cavity while others make their first appearance in distant organs like the lung or brain. In such cases, only the theoretical knowledge of the highly variable symptomatology of choriocarcinoma combined with the patient's clinical history will arouse suspicion of the disease.

Surgery which was given to the majority of the patients in the present series, has now been abandoned as the primary treatment, at least as far as fertile patients are concerned. Therefore, the diagnosis of a trophoblastic tumour is rarely established histologically today. A tentative diagnosis made on the basis of curettings and/or clinical investigations is substantiated by the demonstration of HCG and the exclusion of pregnancy. Reasonable attempts should be made to establish the spread of the disease (pelvic arteriography, chest radiography, tomography, brain scanning, HCG determination in spinal fluid, etc.). As far as treatment is concerned, it matters little whether a post-molar patient with a diagnosis of invasively growing trophoblastic tumour harbours an invasive mole or a choriocarcinoma. On the other hand, for scientific purposes and in the comparison of therapeutic results, this is unsatisfactory. If no definite diagnosis can be made, the case should be recorded, e.g. as a post-molar trophoblastic tumour.

The standard treatment of trophoblastic tumours is cancer chemotherapy, the drug of choice is Methotrexate alone or combined with other cytostatics. There is no agreement as to how long persistent HCG excretion should be tolerated after molar pregnancy before treatment is started; nor can the dosage level or the number of treatment courses be generally outlined. It is also uncertain to what extent attention should be focused on the ABO and HL A types of the patient and her husband and on the intensity of cellular reaction to the tumour when histological specimens are available. Finally the influence of the immunosuppressive side effect of intensive chemotherapy in relation to the survival of these allogenic tumours should be considered.

The rarity of the trophoblastic tumours in question and the attendant diagnostic problems, the need for special investigations, which often require advanced techniques, and the problems involved in optimal chemotherapy all strongly indicate the need for centralized treatment. This was organized in Britain about 10 years ago (4) and is still a matter of debate in other European countries. In this country efforts at centralization began in 1967 and at the request of the European Organization for Research on Treatment of Cancer (EORTC) a national choriocarcinoma group was established 3 years later. Today the group has 9 members representing the following specialities: pathology, endocrinology, diagnostic radiology, cancer and transplantation immunology, gynaecology and obstetrics, cancer chemo- and radiotherapy, electron microscopy and tissue culture. In addition to the appraisal of histological specimens made at the request of colleagues since its unofficial establishment, the group has been involved in the investigation of 17 patients who had, or were suspected of having, invasive trophoblastic tumours. Two of these patients died of generalized choriocarcinoma. One (case 28) was briefly reported above, and the other died of a brain metastasis a few days after the possibility of trophoblastic disease was definitely confirmed. The basic condition of the latter patient remained untreated, and she is probably the only patient diagnosed in this country since 1965 who has died of choriocarcinoma.

In countries which still have decentralized treatment, the establishment of choriocarcinoma groups must be an advantage to the patients. Undoubtedly it will lead to improved diagnostic techniques in trophoblastic diseases, a safer differentiation between cases which need treatment and those which do not, and a better planning of treatment and check-ups. From a scientific point of view the establishment of such groups would also mark an advance on past practice. We still do not know the prognosis for the individual chorio-

mole. However in some cases, the diagnosis of the transition of moles into choriocarcinoma was unduly delayed because the follow-up was inadequate by present standards. The determination of urinary HCG levels is particularly important.

Generally frequent measurements of HCG were made during treatment for choriocarcinoma, but often the measurements were performed more sporadically after the patient's discharge from hospital. This applies, in particular to patients from the period when surgery and irradiation were the conventional methods of treatment. Therefore the mean interval between diagnosis and cure in the non-fatal patients cannot be stated with sufficient certainty in this study. This is regrettable both from a clinical as well as a theoretical point of view.

Generally speaking, the dating of the tumour is made with the greatest certainty in primigravidae because malignant trophoblasts can remain latent for years (3). Therefore, in a multigravida the possibility always exists that the choriocarcinoma dates from an earlier pregnancy than the last one. Two patients (cases 19 and 47) had a latent interval of about 8 and 5 years, respectively between the removal of a hydatidiform mole and the diagnosis of choriocarcinoma. It cannot be excluded that the malignancy in these patients was derived from a later pregnancy which, in fact, was diagnosed in case 19 (abortion). On the assumption that a mole occurs once in every 1 300 births (10) and choriocarcinoma once per 49 000 deliveries (14), the chance that the 2 types of trophoblastic diseases will occur independently in the same patient is negligible.

The over-all mortality in this series was 64% (32 patients). Eight patients (16%) recovered from localized and 10 (20%) from generalized disease. Among 16 patients registered during the first half of the period under investigation, 3 (19%) survived, as compared with 15/34 (44%) registered during the second half. Referring to generalized cases exclusively the corresponding figures are 2/14 (14%) and 8/27 (30%). These results may seem surprising: several patients received little treatment and only 5 of the survivors were given chemotherapy. However as all the diagnoses have recently been reappraised (14), there is only a slight risk that trophoblastic lesions other than choriocarcinoma have been included.

The therapy used varied so much from case to case that no regimen given can be stated to be superior to another. With this reservation in mind, the following conclusions can be drawn from this series: (1) no essential difference was found between the fatal and non-fatal cases in respect of the mean interval from the antecedent pregnancy to diagnosis, (2) the number and the outcome of pregnancies in multigravidae prior to the diagnosis seemed to be without prognostic significance (3) patients suffering from generalized trophoblastic disease at the time of diagnosis had a very grave prognosis as compared with localized cases; (4) a positive correlation was found between young age at the time of diagnosis and a more favourable prognosis. These observations may not be confirmed (in full) in prospective series given cancer chemotherapy exclusively.

In an attempt to find factors which may influence the course of the disease we have been interested in the transplantation immunology of choriocarcinoma during recent years (9 12). At present, there are indications that a tumour which is incompatible with the patient in respect of the transplantation antigens (the ABO and HL A systems) responds better to treatment than a compatible one. The evaluation of degrees of compatibility between tumour and patient, however is much easier than predicting whether the patient will survive the implanted fetal tumour. The innate ability of the patient to respond to foreign antigens—which, inter alia presumably varies with age—may be of importance just like the depression of the immunological activity which often follows cancer chemotherapy. This problem will be discussed in greater detail in a subsequent paper (13).

Recently Elson described a band of mononuclear cells around invading malignant trophoblast in several cases of choriocarcinoma (5). This finding was interpreted as an attempt at tumour rejection and it was concluded that immunological mechanisms may play a part in the natural history of trophoblastic malignancy. On the basis of the results of a similar investigation including 23 choriocarcinomata removed before any treatment was given (15) we agree with Elston's points of view. We find, too that the specificity of the antigens in the malignant trophoblasts deserves further investigation.

Based on this survey of past practice it would

be reasonable to end up with some current considerations about problems related to invasive mole and choriocarcinoma.

Intensive and prolonged observation of patients who have had a hydatidiform mole will allow a diagnosis of invasive mole and choriocarcinoma to be made earlier than seen in this study. Many more difficulties are involved in the early diagnosis of choriocarcinoma following abortion or delivery. The occurrence of irregular and profuse vaginal bleeding should arouse suspicion of a primary tumour provided that the result of curettage is inconclusive. However some choriocarcinomata develop deep in the myometrium without causing any bleeding into the uterine cavity while others make their first appearance in distant organs like the lung or brain. In such cases, only the theoretical knowledge of the highly variable symptomatology of choriocarcinoma combined with the patient's clinical history will arouse suspicion of the disease.

Surgery which was given to the majority of the patients in the present series, has now been abandoned as the primary treatment, at least as far as fertile patients are concerned. Therefore, the diagnosis of a trophoblastic tumour is rarely established histologically today. A tentative diagnosis made on the basis of curettings and/or clinical investigations is substantiated by the demonstration of HCG and the exclusion of pregnancy. Reasonable attempts should be made to establish the spread of the disease (pelvic arteriography, chest radiography, tomography, brain scanning, HCG determination in spinal fluid, etc.). As far as treatment is concerned, it matters little whether a post-molar patient with a diagnosis of invasively growing trophoblastic tumour harbours an invasive mole or a choriocarcinoma. On the other hand, for scientific purposes and in the comparison of therapeutic results, this is unsatisfactory. If no definite diagnosis can be made, the case should be recorded, e.g., as a post-molar trophoblastic tumour.

The standard treatment of trophoblastic tumours is cancer chemotherapy, the drug of choice is Methotrexate alone or combined with other cytostatics. There is no agreement as to how long persistent HCG excretion should be tolerated after a molar pregnancy before treatment is started, nor can the dosage level or the number of treatment courses be generally outlined. It is also uncertain to what extent attention should be focused on the ABO and HL A types of the patient and her husband and on the intensity of cellular reaction to the tumour when histological specimens are available. Finally the influence of the immunosuppressive side effect of intensive chemotherapy in relation to the survival of these allogeneic tumours should be considered.

The rarity of the trophoblastic tumours in question and the attendant diagnostic problems, the need for special investigations, which often require advanced techniques, and the problems involved in optimal chemotherapy all strongly indicate the need for centralized treatment. This was organized in Britain about 10 years ago (4) and is still a matter of debate in other European countries. In this country efforts at centralization began in 1967 and at the request of the European Organization for Research on Treatment of Cancer (EORTC) a national choriocarcinoma group was established 3 years later. Today the group has 9 members representing the following specialities: pathology, endocrinology, diagnostic radiology, cancer and transplantation immunology, gynaecology and obstetrics, cancer chemo- and radiotherapy, electron microscopy and tissue culture. In addition to the appraisal of histological specimens made at the request of colleagues since its unofficial establishment, the group has been involved in the investigation of 17 patients who had, or were suspected of having, invasive trophoblastic tumours. Two of these patients died of generalized choriocarcinoma. One (case 28) was briefly reported above, and the other died of a brain metastasis a few days after the possibility of trophoblastic disease was definitely confirmed. The basic condition of the latter patient remained untreated, and she is probably the only patient diagnosed in this country since 1965 who has died of choriocarcinoma.

In countries which still have decentralized treatment, the establishment of choriocarcinoma groups must be an advantage to the patients. Undoubtedly it will lead to improved diagnostic techniques in trophoblastic diseases, a safer differentiation between cases which need treatment and those which do not, and a better planning of treatment and check-ups. From a scientific point of view the establishment of such groups would also mark an advance on past practice. We still do not know the prognosis for the individual chorio-

mole However in some cases, the diagnosis of the transition of moles into choriocarcinoma was unduly delayed because the follow-up was inadequate by present standards. The determination of urinary HCG levels is particularly important.

Generally frequent measurements of HCG were made during treatment for choriocarcinoma, but often the measurements were performed more sporadically after the patient's discharge from hospital. This applies, in particular to patients from the period when surgery and irradiation were the conventional methods of treatment. Therefore the mean interval between diagnosis and cure" in the non-fatal patients cannot be stated with sufficient certainty in this study This is regrettable both from a clinical as well as a theoretical point of view

Generally speaking, the dating of the tumour is made with the greatest certainty in primigravidae because malignant trophoblasts can remain latent for years (3). Therefore, in a multigravida the possibility always exists that the choriocarcinoma dates from an earlier pregnancy than the last one Two patients (cases 19 and 47) had a latent interval of about 8 and 5 years, respectively between the removal of a hydatidiform mole and the diagnosis of choriocarcinoma. It cannot be excluded that the malignancy in these patients was derived from a later pregnancy which in fact, was diagnosed in case 19 (abortion). On the assumption that a mole occurs once in every 1 300 births (10) and choriocarcinoma *once per 49 000 deliveries (14) the chance that* the 2 types of trophoblastic diseases will occur independently in the same patient is negligible.

The over-all mortality in this series was 64% (32 patients). Eight patients (16%) recovered from localized and 10 (20%) from generalized disease Among 16 patients registered during the first half of the period under investigation, 3 (19%) survived, as compared with 15/34 (44%) registered during the second half Referring to generalized cases exclusively the corresponding figures are 2/14 (14%) and 8/-7 (30%). These results may seem surprising- several patients received little treatment and only 5 of the survivors were given chemotherapy However as all the diagnoses have recently been reappraised (14) there is only a slight risk that trophoblastic lesions other than choriocarcinoma have been included.

The therapy used varied so much from case to case that no regimen given can be stated to be superior to another With this reservation in mind, the following conclusions can be drawn from this series: (1) no essential difference was found between the fatal and non-fatal cases in respect of the mean interval from the antecedent pregnancy to diagnosis, (?) the number and the outcome of pregnancies in multigravidae prior to the diagnosis seemed to be without prognostic significance (3) patients suffering from generalized trophoblastic disease at the time of diagnosis had a very grave prognosis as compared with localized cases, (4) a positive correlation was found between young age at the time of diagnosis and a more favourable prognosis. These observations may not be confirmed (in full) in prospective series given cancer chemotherapy exclusively

In an attempt to find factors which may influence the course of the disease we have been interested in the transplantation immunology of choriocarcinoma during recent years (9 1). At present, there are indications that a tumour which is incompatible with the patient in respect of the transplantation antigens (the ABO and HL A systems) responds better to treatment than a compatible one. The evaluation of degrees of compatibility between tumour and patient however is much easier than predicting whether the patient will survive the implanted fetal tumour The innate ability of the patient to respond to foreign antigens—which inter alia, presumably varies *with age—may be of importance just like the* depression of the immunological activity which often follows cancer chemotherapy This problem will be discussed in greater detail in a subsequent papter (13).

Recently Elson described a band of mononuclear cells around invading malignant trophoblast in several cases of choriocarcinoma (5). This finding was interpreted as an attempt at tumour rejection, and it was concluded that immunological mechanisms may play a part in the natural history of trophoblastic malignancy On the basis of the results of a similar investigation including 23 choriocarcinomata removed before any treatment was given (15), we agree with Elston's points of view We find, too that the specificity of the antigens in the malignant trophoblasts deserves further investigation.

Based on this survey of past practice it would

Acta Obstet Gynec Scand 52. 221–228 1973

CONTRACEPTIVE TREATMENT WITH LOW DOSES OF GESTAGEN IN CASES WITH MEDICAL HISTORY OF HEPATOSIS OF PREGNANCY

Pekka Ylöstalo and Salme Vuopala

From the Department of Obstetrics and Gynecology (Head. Prof. P. A. Järvinen), University of Oulu, Oulu, Finland

Abstract. The effect of low-dose gestagen pills on hepatic function was investigated in 5 subjects who had previously suffered from hepatosis of pregnancy and 9 healthy controls. The gestagen used was 0.3 mg of norethisterone daily. The following laboratory tests were performed during the 6 months of low-dose gestagen medication: Serum alanine aminotransferase (S-ALAT-GPT), serum cholesterol, triglycerides, total bilirubin, total proteins, electrophoretic distribution of proteins, α-1-antitrypsin, ceruloplasmin, acid α-1-glycoprotein, α-2-HS-glycoprotein, total haptoglobin, β-lipoproteins, α-2-macroglobulin, transferrin, and IgG immunoglobulins. The tests either revealed no differences between the two groups or showed only minor changes. None of the subjects discontinued the treatment because of hepatic dysfunction or symptoms suggestive of such. The authors consider oral contraceptives containing low doses of gestagen to be suitable even for patients who have suffered from hepatosis of pregnancy.

Hepatosis of pregnancy is a hepatic dysfunction which occurs during pregnancy and has the features of intrahepatic cholestasis. It is regarded as being due to hormonal factors, chiefly the effect of estrogens (2). The dysfunction is characterized by elevated aminotransferases and frequently by a subicteric condition, in addition to which α-2 globulin and β-globulin of the serum proteins are elevated (10, 11 13 25, 31). Serum bile acid level is greatly elevated, which fact is considered significant for the occurrence of itching, the most typical symptom of hepatosis of pregnancy (27). Moreover the prothrombin index may decline if the icterus is of long duration. Intrahepatic cholestasis has similarly been found to have a stimulating effect on hepatic protein synthesis (14). The following proteins, among others, are synthesized in the liver: albumin, α-1-acid-glycoprotein, α-1 antitrypsin, haptoglobin, α-2-macroglobulin, Gc-globulins, prothrombin, proconvertin, transferrin, high and low density lipoproteins, and possibly hematopexin and ceruloplasmin. Immunoglobulins are produced in the reticulo-endothelial tissue, not in the liver (26).

Oral contraceptives, both combined and sequential preparations, may bring about icteric liver disorders (2) particularly in patients who have suffered from hepatosis of pregnancy. Low-dose gestagen has not been found to induce liver dysfunction (15) even in subjects who have previously had hepatosis of pregnancy (20 29).

The purpose of the present work was to elucidate the effects of low-dose oral gestagen on subjects with previous hepatosis of pregnancy and to follow the liver function of such patients.

MATERIAL AND METHODS

The effect of low-dose gestagen pills on the hepatic function of 5 patients who had previously suffered from hepatosis of pregnancy and 9 healthy controls who had not had hepatosis of pregnancy or any hepatic disorders while on other oral contraceptives was investigated. The oral contraceptive used was 0.3 mg of norethisterone (NET) daily. The following laboratory tests were performed during the experiment: serum alanine aminotransferase (S-ALAT-GPT) (21), serum cholesterol (18) and triglycerides (22) prior to the introduction of the pill and after 2, 4, 6 and 8 weeks and 3 and 6 months of treatment. Further tests were performed on total bilirubin (16), total proteins (30), electrophoretic distribution of proteins: albumin, α-1-globulin, α-2-globulin, β-globulin and γ-globulin (9); radial immunodiffusion (17) was used for assaying α-1-antitrypsin, ceruloplasmin, acid α-1-glycoprotein, α-2-HS-glycoprotein, total haptoglobin, α-2-macroglobulin, transferrin and IgG immunoglobulin, and β-lipoproteins (4) before the initiation of contraceptive medication and after 4 weeks, 3 and 6 months. One of the patients who had had hepatosis of pregnancy gave up

carcinoma patient nor can we conclusively ex plain why just choriocarcinoma can be cured with cancer chemotherapeutic agents. It may well be that the solution to these problems may introduce new perspectives in the therapy of other malignant neoplasms.

ACKNOWLEDGEMENTS

The author is indebted to Professor M Ingerslev University of Aarhus, for his sober criticism and helpful suggestions, and to the clinicians who permitted me to make use of case records in this study

REFERENCES

1. Acosta-Sison, H. Can the implanting trophoblasts of the fertilized ovum develop immediately into chorionepithelioma? Amer J Obstet Gynec 69 442, 1955
2. Bagshawe, K. D In Choriocarcinoma, p 61 Edward Arnold (Publishers) Ltd., London, 1969
3. Dyke, P C. & Fink, L. M.: Latent choriocarcinoma. Cancer 20 150, 1967
4. Edgar Laboratory Fulham Hospital, St. Dunstans Road, London W.6, England (Head. Professor K. D Bagshawe).
5. Elston, C. W. Cellular reaction to choriocarcinoma. J Path 97 261 1969
6. Hertig, A. T & Mansell, H. Tumours of the female sex organs. Part I. Hydatidiform mole and choriocarcinoma. Atlas of Tumour Pathology sect. 9 fasc. 33. Armed Forces Institute of Pathology Washington, D C., 1956.
7. Hertig, A. T & Sheldon, W. H. Hydatidiform mole, a pathologico-clinical correlation of 200 cases. Amer J Obstet Gynec 53 1 1947
8. Homburger F & Schwartz, R. S. (eds.) Immunological aspects of neoplasia. Progress in Experimental Tumor Research, vol. 13 S. Karger Basel, München and New York, 1970.
9. Kissmeyer Nielsen, F & Thorsby E. Human transplantation antigens. Transplant Rev 4 103 1970.
10. Kolstad, P & Hognestad, J Trophoblastic tumours in Norway Acta Obstet Gynec Scand 44 80 1965
11. Li, M. C., Hertz, R. & Spencer D. B.: Effect of methotrexate therapy upon choriocarcinoma and chorioadenoma. Proc Soc Exp Biol Med 93 361, 1956.
12. Mogensen, B. & Kissmeyer-Nielsen, F Current data on HL-A and ABO typing in gestational choriocarcinoma and invasive mole. Transplant Proc 3 1267 1971.
13. Mogensen, B. and Kissmeyer-Nielsen, F Gestational choriocarcinoma, invasive mole and transplantation antigens (to appear in Ser Haemat).
14. Mogensen, B. & Olsen, S. Gestational choriocarcinoma in Denmark, 1940-1969 A reappraisal based on modern histologic criteria. Acta Obstet Gynec Scand 51 63 1972.
15. Mogensen, B. & Olsen, S. Cellular reaction to gestational choriocarcinoma and invasive mole (in preparation).
16. Ober W B., Edgcomb, J H. & Price, E. B., Jr The pathology of choriocarcinoma. Ann NY Acad Sci 17 art. 10: 301 302, 1971.
17. Ober W B., Edgcomb, J H. & Price E. B., Jr The pathology of choriocarcinoma. Ann NY Acad Sci 172 art. 10: 307 1971
18. Ober, W B., Edgcomb, J H. & Price, E. B., Jr The pathology of choriocarcinoma. Ann NY Acad Sci 172 art. 10: 310, 1971
19. Park, W W.: In Choriocarcinoma, pp. 87-90. William Heinemann Medical Books Limited, London, 1971.
20. Ringertz, N Hydatidiform mole invasive mole and choriocarcinoma in Sweden 1958-1965. Acta Obstet Gynec Scand 49 195 1970.
21. The Danish Cancer Registry under the National Anti-Cancer League, Strandboulevarden 49 DK 2100 Copenhagen Ø Denmark (Head. Dr J Clemmesen).
22. Weiss, D W (ed.): Immunological Parameters of Host Tumor Relationships. Academic Press, New York and London, 1971

Submitted for publication May 29 197

Bent Mogensen
Cancer Research Institute
Radiumstationen
Aarhus
Denmark

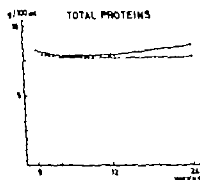


Fig. 5. Serum total proteins before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy ····· the healthy control group.

Serum total bilirubin (Fig. 4). The hepatosis group had one elevated value at one month (1.5 mg/100 ml) while the control group had no elevated values. The high values of the hepatosis group were normal again in the control test. At one month in the control group and 6 months in the hepatosis group the average level was somewhat higher than the 0-test level, the differences were almost significant ($p < 0.05$).

Serum total proteins (Fig. 5). The mean value of the hepatosis group was significantly ($p < 0.01$) higher than that of the control group at 6 months. In the control group the value at 6 months was significantly ($p < 0.01$) lower than that recorded in the 0-test. The hepatosis group had 4 elevated values in the 0-test (8.3, 8.3, 8.6, 8.6 g/100 ml), one at 4 weeks (8.4 g/100 ml), one at 3 months (8.9 g/100 ml), and 2 at 6 months (8.6, 9.1 g/100 ml). The control group had 3 elevated values in the 0-test (8.3, 8.4, 8.7 g/100 ml) and 2 at 4 weeks (8.3, 8.7 g/100 ml).

Serum albumin (Fig. 6). The mean value of the hepatosis group at 4 weeks was significantly ($p < 0.01$) lower than the corresponding value of the control group and almost significantly ($p < 0.05$) lower than the value obtained in the 0-test. During the contraceptive medication the hepatosis group had one declined value at 4 weeks (45.6 %).

Serum α-1-globulin (Fig. 7). No significant differences were noted between the groups or the different points of time. The hepatosis group had one reduced value at 3 months (1.8 %), while the control group had one at 3 months and another at 6 months (1.3 % and 1.7 %).

Serum α-2-globulin (Fig. 8). No significant differences were to be seen either between the two groups or between the different times at which tests were performed in each group. Single ele-

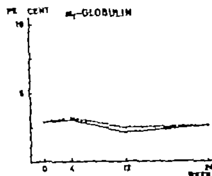


Fig. 7. Serum α1-globulin before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy ····· the healthy control group.

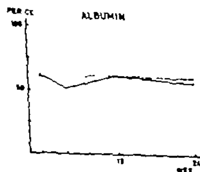


Fig. 6. Serum albumin before (O) and during the use of norethisterone (NET 0.3 mg). —, The group with previous hepatosis of pregnancy ····· the healthy control group.

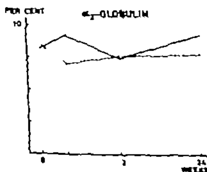


Fig. 8. Serum α2-globulin before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy ····· the healthy control group.

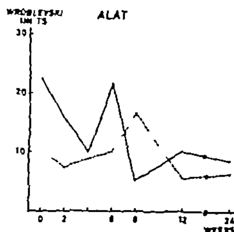


Fig 1 Serum alanine aminotransferase (ALAT/GPT) before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy the healthy control group.

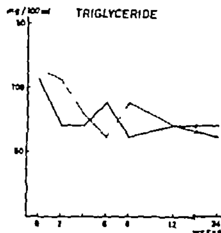


Fig 3 Serum triglycerides before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy -, the healthy control group.

the "mini-pill" after 3 months because of a holiday and another did so after 6 months because of oligomenorrhoea. One of the controls gave up the pill after one month, 2 after 3 months, and one after 6 months, most because of menstrual irregularities. None of the subjects discontinued medication because of hepatic disorders or symptoms suggestive of such.

RESULTS

Serum alanine aminotransferase (S-ALAT–GPT) (Fig. 1). The average ALAT (GPT) values at 3 months were almost significantly higher in the group with hepatosis than in the control group ($p < 0.05$). Single elevated values were noted in the hepatosis group at 2 and 6 weeks (44 and 62 Wroblevski units) but they were normal again by the time of the following control test. Occasional elevated values were also encountered in the control group at 4, 6 and 8 weeks (39, 44, 63 Wroblevski units) but these similarly returned to normal before the control test.

Serum cholesterol (Fig. 2). At 6 months the hepatosis group had values which were on the average significantly higher than those of the *control group ($p < 0.01$). No pathological values* were recorded in either group.

Serum triglycerides (Fig. 3). At 8 weeks the mean value of the hepatosis group was significantly lower than that of the control group. Both groups showed a slight declining tendency if compared with the test recorded before the introduction of the pill (0-test) the decline was almost significant in the control group at 3 months ($p < 0.05$). Pathologically high values were not noted *except in one woman of the control group after 2* weeks of medication.

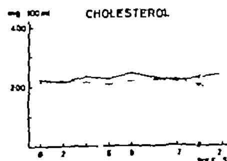


Fig 2 Serum cholesterol before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy the healthy control group.

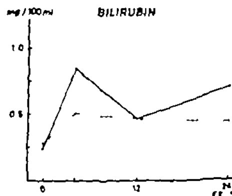


Fig 4 Serum total bilirubin before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy the healthy control group

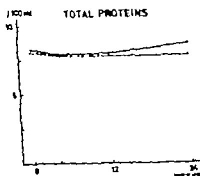


Fig. 5. Serum total proteins before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy; ...... the healthy control group.

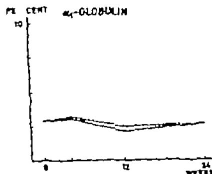


Fig. 7. Serum α_1-globulin before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy; ...... the healthy control group.

Serum total bilirubin (Fig. 4). The hepatosis group had one elevated value at one month (1.5 mg/100 ml), while the control group had no elevated values. The high values of the hepatosis group were normal again in the control test. At one month in the control group and 6 months in the hepatosis group the average level was somewhat higher than the 0-test level, the differences were almost significant ($p < 0.05$).

Serum total proteins (Fig. 5). The mean value of the hepatosis group was significantly ($p < 0.01$) higher than that of the control group at 6 months. In the control group the value at 6 months was significantly ($p < 0.01$) lower than that recorded in the 0-test. The hepatosis group had 4 elevated values in the 0-test (8.3, 8.3, 8.6, 8.6 g/100 ml), one at 4 weeks (8.4 g/100 ml), one at 3 months (8.9 g/100 ml), and 2 at 6 months (8.6, 9.1 g/100 ml). The control group had 3 elevated values in the 0-test (8.3, 8.4, 8.7 g/100 ml) and 2 at 4 weeks (8.3, 8.7 g/100 ml).

Serum albumin (Fig. 6). The mean value of the hepatosis group at 4 weeks was significantly ($p < 0.01$) lower than the corresponding value of the control group and almost significantly ($p < 0.05$) lower than the value obtained in the 0-test. During the contraceptive medication the hepatosis group had one declined value at 4 weeks (45.6%).

Serum α-1-globulin (Fig. 7). No significant differences were noted between the groups or the different points of time. The hepatosis group had one reduced value at 3 months (1.8%), while the control group had one at 3 months and another at 6 months (1.3% and 1.7%).

Serum α-2-globulin (Fig. 8). No significant differences were to be seen either between the two groups or between the different times at which tests were performed in each group. Single ele-

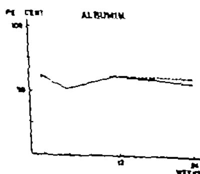


Fig. 6. Serum albumin before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy; ...... the healthy control group.

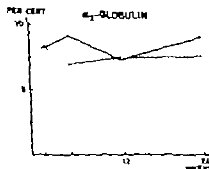


Fig. 8. Serum α_2-globulin before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy; ...... the healthy control group.

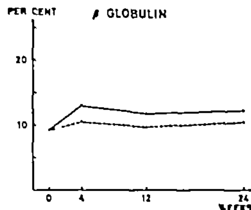


Fig 9 Serum β-globulin before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy· the healthy control group.

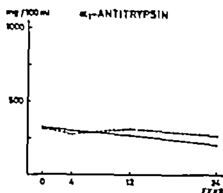


Fig 11 Serum α_1-antitrypsin before (O) and during the use of norethisterone (NET 0.3 mg) — The group with previous hepatosis of pregnancy· the healthy control group.

vated values were recorded for the hepatosis group at 4 weeks (10.7%) and 6 months (11.3%) reduced values were noted in the control group at 4 weeks (1 0%) *and 6 months* (4.3%).

Serum β-globulin (Fig. 9) The mean value of the hepatosis group was almost significantly ($p <$ 0.05) higher than that of the control group at 3 and 6 months, and significantly ($p < 0.01$) higher than the value of the 0-test at 6 months. Single elevated values were recorded at 4 weeks and 3 and 6 months (21 0 14 8 12.8%) in the hepatosis group and at 6 months (13.5%) in the control group The hepatosis group had one reduced value in the 0-test (6.7%) and another at 4 weeks (7 0%) while the control group had one in the 0-test (3 7%)

Serum γ-globulin (Fig. 10) *No significant dif* ferences were noted either between the groups or the different points of time within the groups. The control group had 2 elevated values both in the 0-test (25 ? 26.5%) and at 3 months (?4 0). The hepatosis group had 2 elevated values at 4 weeks (28.0 28.9%).

α-1-antitrypsin (Fig. 11). The mean value of the hepatosis group at 3 months was significantly ($p < 0\ 01$) lower than that of the control group At 6 months both the hepatosis group and the control group had one reduced value (104 and 150 mg/100 ml, respectively)

Ceruloplasmin (Fig. 12). The mean value of the hepatosis group was almost significantly ($p <$ 0 05) lower at 3 months than in the 0-test. N other noticeable differences were seen. In the 0-test both the hepatosis group and the control group had one elevated value (41 2 and 40 mg/ *100 ml, respectively). The hepatosis group* had reduced values in the 0-test (16.5 6.5 mg/100

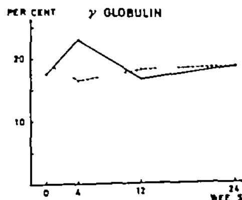


Fig 10 Serum γ-globulin before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis *of pregnancy* · the healthy control group.

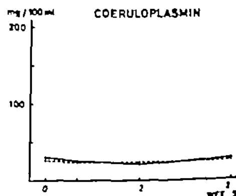


Fig 12 Serum ceruloplasmin before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy the healthy control group.

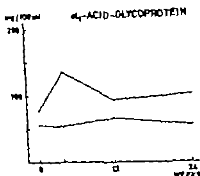


Fig. 11. Serum α_1-acid-glycoprotein before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy ······ the healthy control group.

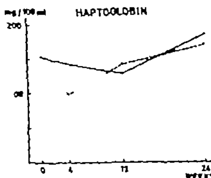


Fig. 15. Serum haptoglobin before (O) and during the use of Norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy ······ the healthy control group.

ml), 2 at 4 weeks (16, 25 mg/100 ml) 2 at 3 months (18 23.5 mg/100 ml) and one at 6 months (25 mg/100 ml), while the control group had 6 reduced values in the 0-test (18–26 mg/100 ml), 4 at 4 weeks (18–26 mg/100 ml) 4 at 3 months (16–26.5 mg/100 ml), and 4 at 6 months (21.5–26.5 mg/100 ml).

α-1-acid glycoprotein (Fig. 13). The values of the hepatosis group in the 0-test and at 6 months were on the average almost significantly ($p <$ 0.05) higher than the corresponding values of the control group. One elevated value was recorded in the hepatosis group at 4 weeks (270 mg/100 ml). The hepatosis group had one reduced value in the 0-test (52 mg/100 ml) and one at 4 weeks (40 mg/100 ml), while the control group had 5 reduced values in the 0-test (26–54 mg/100 ml), one at 4 weeks (51 mg/100 ml) and 2 at 6 weeks (31 46 mg/100 ml).

α-2-HS-glycoprotein (Fig. 14). The two groups displayed no significant differences during the contraceptive medication. Before the introduction of the pill the mean value of the hepatosis group was significantly ($p < 0.01$) higher than that of the control group. The hepatosis group had one elevated value at 4 weeks (117 mg/100 ml) while the control group had 2 at that time (87 and 104 mg/100 ml).

Total haptoglobin (Fig. 15). No significant differences between the groups were noted. The hepatosis group had one elevated value at 6 months (230 mg/100 ml). The control group had one elevated value in the 0-test (240 mg/100 ml), one at 3 months (248 mg/100 ml) and one at 6 months (380 mg/100 ml), and one reduced value at 4 weeks (10 mg/100 ml) and 3 months (24 mg/100 ml).

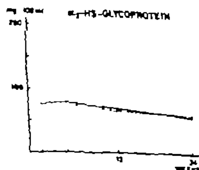


Fig. 14. Serum α_2-HS-glycoprotein before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy ······ the healthy control group.

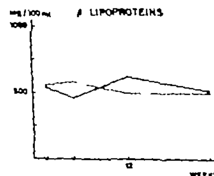


Fig. 16. Serum β-lipoproteins before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy ······ the healthy control group.

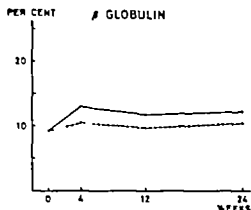


Fig 9 Serum β-globulin before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy the healthy control group.

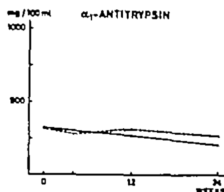


Fig 11 Serum α_1-antitrypsin before (O) and during the use of norethisterone (NET 0.3 mg) — The group with previous hepatosis of pregnancy the healthy control group.

vated values were recorded for the hepatosis group at 4 weeks (10 7%) and 6 months (11.3%) reduced values were noted in the control group at 4 weeks (1 0%) and 6 months (4 3%).

Serum β-globulin (Fig. 9) The mean value of the hepatosis group was almost significantly ($p <$ 0 05) higher than that of the control group at 3 and 6 months, and significantly ($p <$ 0 01) higher than the value of the 0-test at 6 months. Single elevated values were recorded at 4 weeks and 3 and 6 months (21 0 14 8 12.8%) in the hepatosis group and at 6 months (13.5 %) in the control group. The hepatosis group had one reduced value in the 0-test (6.7%) and another at 4 weeks (7 0%), while the control group had one in the 0-test (3 7%).

Serum γ-globulin (Fig. 10) No significant differences were noted either between the groups or the different points of time within the groups. The control group had 2 elevated values both in the 0-test (25.2, 26.5%) and at 3 months (?4 0 %). The hepatosis group had 2 elevated values at 4 weeks (28 0 28.9%)

α-1-antitrypsin (Fig. 11). The mean value of the hepatosis group at 3 months was significantly ($p <$ 0 01) lower than that of the control group. At 6 months both the hepatosis group and the control group had one reduced value (104 and 150 mg/100 ml, respectively)

Ceruloplasmin (Fig. 12). The mean value of the hepatosis group was almost significantly ($p <$ 0 05) lower at 3 months than in the 0-test. No other noticeable differences were seen. In the 0-test both the hepatosis group and the control group had one elevated value (41.2 and 40 mg/100 ml respectively). The hepatosis group had reduced values in the 0-test (16.5 6 5 mg/100

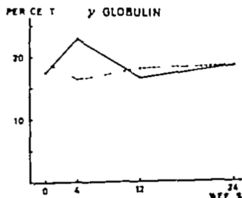


Fig 10 Serum γ-globulin before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy the healthy control group.

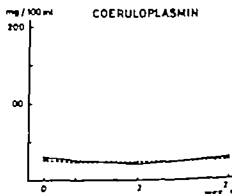


Fig 12 Serum ceruloplasmin before (O) and during the use of norethisterone (NET 0.3 mg) — The group with previous hepatosis of pregnancy , the healthy control group.

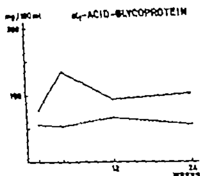


Fig. 13. Serum α1-acid-glycoprotein before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy- - - the healthy control group.

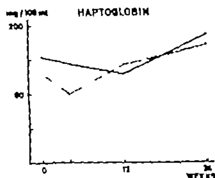


Fig. 15. Serum haptoglobin before (O) and during the use of Norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy - - -, the healthy control group.

ml), 2 at 4 weeks (16, 25 mg/100 ml) 2 at 3 months (18, 23.5 mg/100 ml) and one at 6 months (25 mg/100 ml), while the control group had 6 reduced values in the 0-test (18–26 mg/100 ml), 4 at 4 weeks (18–26 mg/100 ml) 4 at 3 months (16–26.5 mg/100 ml) and 4 at 6 months (21.5–26.5 mg/100 ml).

α-1-acid glycoprotein (Fig. 13). The values of the hepatosis group in the 0-test and at 6 months were on the average almost significantly ($p < 0.05$) higher than the corresponding values of the control group. One elevated value was recorded in the hepatosis group at 4 weeks (270 mg/100 ml). The hepatosis group had one reduced value in the 0-test (52 mg/100 ml) and one at 4 weeks (40 mg/100 ml), while the control group had 5 reduced values in the 0-test (26–54 mg/100 ml), one at 4 weeks (51 mg/100 ml) and 2 at 6 weeks (31, 46 mg/100 ml).

α-2 HS-glycoprotein (Fig. 14). The two groups displayed no significant differences during the contraceptive medication. Before the introduction of the pill the mean value of the hepatosis group was significantly ($p < 0.01$) higher than that of the control group. The hepatosis group had one elevated value at 4 weeks (117 mg/100 ml), while the control group had 2 at that time (87 and 104 mg/100 ml).

Total haptoglobin (Fig. 15). No significant differences between the groups were noted. The hepatosis group had one elevated value at 6 months (230 mg/100 ml). The control group had one elevated value in the 0-test (240 mg/100 ml), one at 3 months (248 mg/100 ml) and one at 6 months (380 mg/100 ml) and one reduced value at 4 weeks (10 mg/100 ml) and 3 months (24 mg/100 ml).

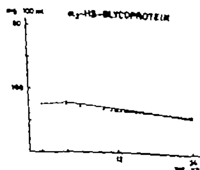


Fig. 14. Serum α2-HS-glycoprotein before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy- - - the healthy control group.

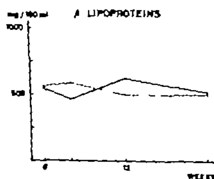


Fig. 16. Serum β-lipoproteins before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy- - - the healthy control group.

13 731043—

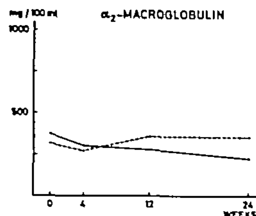


Fig 17 Serum α_2-macroglobulin before (O) and during the use of norethisterone (NET 0.3 mg) — The group with previous hepatosis of pregnancy the healthy control group.

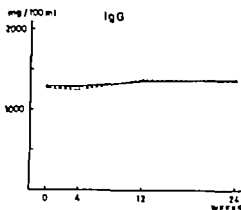


Fig 19 Serum IgG before (O) and during the use of norethisterone (NET 0.3 mg). — The group with previous hepatosis of pregnancy the healthy control group

β-lipoproteins (Fig. 16). The mean value of the hepatosis group at 3 months was almost significantly ($p < 0.05$) higher than that of the control group. The hepatosis group had one elevated value in the 0-test (670 mg/100 ml) and at 3 months (665 mg/100 ml), and one reduced value at 4 weeks (110 mg/100 ml). The control group had one elevated value in the 0-test (665 mg/100 ml) one at 4 weeks (803 mg/100 ml) and 2 at 3 months (669 675 mg/100 ml).

α-2-macroglobulin (Fig. 17) The mean value of the hepatosis group was significantly ($p < 0.01$) lower than that of the control group at 3 and 6 months. The hepatosis group had one elevated value in the 0-test (680 mg/100 ml), and the control group had 2 at 3 months (435 435 mg/100 ml)

Transferrin (Fig. 18). The control group had

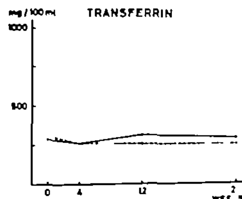


Fig 18 Serum transferrin before (O) and during the use of norethisterone (NET 0.3 mg) — The group with previous hepatosis of pregnancy the healthy control group.

the value almost significantly lower at 6 months than in the 0-test. The hepatosis group had one elevated value (415 mg/100 ml) and 2 reduced ones (175 190 mg/100 ml) in the 0-test. The control group had one elevated value in the 0-test (420 mg/100 ml) and _ reduced values at 3 months (140 186 mg/100 ml)

IgG (Fig 19). No significant differences were noted between the groups or the different points of time. Neither elevated nor reduced values were recorded

DISCUSSION

Serum alanine aminotransferase (ALAT GPT) values have been found to increase during the use of combined oral contraceptives, particularly following the introduction of medication (1) whereas no great increase in ALAT activity has been noted during the use of low-dose gestagen pills (15). The present investigation revealed or 3 somewhat elevated values in both the hepatosis group and the control group which had however returned to normal by the time of the following control test

The values for cholesterol and triglycerides rise during the use of combined oral contraceptives, but not during the consumption of low-dose gestagen (3 15 _3) The effect of oral contraceptives on lipid metabolism has, consequently been attributed to the estrogen component. The present hepatosis group had no pathologically high values for either cholesterol or triglycerides.

Combined oral contraceptives have been found to effect a rise in the values of carrier proteins,

such as transcortin, thyroxine-binding protein transferrin and ceruloplasmin, similar to the rise occurring during pregnancy (1 5 7). During both pregnancy (24) and the use of combined oral contraceptives α-2-macroglobulin values have been found to rise, which rise, owing to the antiplasmin effect of α-2-macroglobulin, has been associated with the increased susceptibility to thrombosis (12) in the same way as the elevated α-1-antitrypsin and β-lipoprotein levels (28). β-lipoproteins also rise in obstructive hepatic diseases (19). Transferrin values decline during hepatic diseases and chronic infections (19). α-1 antitrypsin values may rise in hepatitis and liver cirrhosis, and α-2-macroglobulin values may similarly rise in severe liver diseases (26). Haptoglobin values are elevated during infections and degenerative diseases and decline in serious liver diseases (19). α-1-acid-glycoprotein may be elevated during infections and degenerative diseases.

The elevation of α-globulin and β-globulin values noted during pregnancy is emphasized in patients with hepatosis (11 31). The changes noted in serum protein during "progestagen-only" contraception have been insignificant (6, 8). Comparison of the findings of serum proteins in the hepatosis group and the control group of the present study reveals a few small differences. The values for total proteins were higher in the hepatosis group at 6 months than they were in the control group, and there was an increase in β-globulin values, change similar in direction to the one occurring during hepatosis of pregnancy (11 31) Among the single protein changes we might point out the decline of the α-1-antitrypsin and α-2-macroglobulin values in the hepatosis group during the consumption of gestagen contraceptives

The investigation carried out provides no clear indication that low-dose gestagen pills, in this case 0 3 mg of norethisterone daily would cause hepatic dysfunction even in patients who have previously suffered from hepatosis of pregnancy It is true, of course that the meaning of certain slight differences between the groups is difficult to estimate owing to the small size of the series, and the question therefore requires further elucidation It seems apparent, however that low dose gestagen minipills do not disturb hepatic function even in patients who have had hepatosis of pregnancy and that the "mini-pill" is clearly different from the combined contraceptive pill in this respect. It is naturally advisable to follow hepatic function whenever mini-pills are prescribed for a patient who has previously suffered from hepatosis of pregnancy at least ALAT (GPT), serum total bilirubin and protein electrophoresis should be checked at fairly short intervals in the beginning and every 3–6 months later on.

ACKNOWLEDGEMENTS

We wish to thank Astra-Syntex for the NET 0 3 mg pills placed at our disposal and the payment of the laboratory expenses as well as the Sigfrid Juselius Foundation for financial support during the analysis of the data.

REFERENCES

1 Adlercreutz, H., Eliasson, A., Helve, A., Luukkainen, T Penttilä, I. & Saukkonen, H. Investigation on the effect of an oral contraceptive and its components on liver function, serum proteins, copper, coeruloplasmin and γ-glutamyl peptidase in postmenopausal women. *Scand J Gastroenter 3* 273 1968.

2. Adlercreutz, H. & Tenhunen, R. Some aspects of the interaction between natural and synthetic female sex hormones and the liver. Amer J Med 49 630, 1970.

3 Barton, G. M. G., Freeman, P R. & Lawson, J P Oral contraceptives and serum lipids. *J Obstet Gynaec Brit Commw 77* 551, 1970.

4. Burstein, M. & Samaille, J Dosage des β-lipoprotéines sériques après précipitation sélective par l'héparine. *Presse Med 66*. 974 1958.

5. Editorial: Plasma-proteins and oral contraceptives. *Lancet 1* 72, 1970

6. Editorial: Progestagen-only contraception. *Lancet 1* 25, 1971.

7 Gaffney T J Jacobs, J M. & Powell, C W The influence of oral contraceptives on plasma protein levels Ann Gen Pract, p. 93. Ramsay Ware Publishing Pty Ltd 1971

8 Gilfrich, H. J., Nierchalag, E., Dudeck, J & Overzier C Norethisteronönanthat. Untersuchungen während der Anwendung eines langwirkenden parenteralen Kontrazeptivum. Dtsch Med Wschr 94 2473, 1969

9 Grunbaum, B. W Lyons, M F Carroll, N. V & Zec, J Quantitative analyses of normal human serum proteins on permanently transparentized cellulose acetate membranes. Microchem *J 7* 54 1963

10 Haemmerli, U P Jaundice during pregnancy Acta Med Scand *179* Suppl. 444 1966.

11 Haemmerli, U P & Wyss, H L. Recurrent intrahepatic cholestasis of pregnancy Medicine (Baltimore) 46 299, 1967

12. Horne, C H. W Howie, P W Weir R. J & Goudie R. B Effect of combined oestrogen-progestogen oral contraceptives on serum levels of α_2-macro-

globulin, transferrin, albumin, and IgG Lancet 1 49 1970.
13 Ikonen, I : Jaundice in late pregnancy Acta Obstet Gynec Scand 43 Suppl. 5 1964
14 Kühn, H A. & Richter F: Pathophysiologie und Klinik der intrahepatischen Cholestase Med Klin 65: 1 1970.
15 Larsson-Cohn U: Contraceptive treatment with low doses of gestagens. Clinical experience with daily oral administration of 0.5 mg of chlormadinone acetate and 0.5 0.4 and 0.3 mg of norethindrone Acta Endocrin (Kbh) 64 Suppl. 144 1970.
16. Malloy H T & Evelyn, K. A: The determination of bilirubin with the photoelectric colorimeter J Biol Chem 119 481 1937
17 Mancini G Carbonara, A. O & Heremans, J F Immunochemical quantitation of antigens by single radial immunodiffusion. Int J Immunochem 235 1965
18. Pearson S Stern S & McGavack T A rapid, accurate method for the determination of total cholesterol in serum. Anal Chem 25 813 1953
19 Penny R. The significance of plasma protein determinations to the clinician Ann Gen Pract, Ramsay Ware Publishing Pty Ltd., p. 25 1971
20. Rannevik G Jeppsson, S Kullander S. & Laurell C. B.: Är preventivtabletter kontraindicerade efter genomgången graviditetshepatos? Nord Med 86 986 1971
21 Reitman, S & Frankel, S.: A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 8 56, 1957
22. Royer M I & Ko, H.: A simplified semiautomated assay for plasma triglycerides. Anal Biochem 29 405 1969
23 Rössner S Larsson-Cohn, U Carlson, L. A. & Boberg, J: Effects of an oral contraceptive agent on plasma lipids, plasma lipids, plasma lipoproteins, the intravenous fat tolerance and the post heparin lipoprotein lipase activity Acta Med Scand 190 301 1971
24 Schlagetter K & Schwick, G Immunchemische Untersuchungen der Serumproteine von Schwangeren. Geburtsh Gynäk 157 225 1961
25 Sherlock, S. Jaundice in pregnancy Br Med Bull 4 39 1968.
26 Simmons, P Penny R. & Goller I Plasma proteins. A review Med Aust 2 494 1969
27 Sjövall, J & Sjövall, K. Steroid sulphates in plasma from pregnant women with pruritus and elevated plasma bile acid levels. Ann Clin Res 2 321 1970.
28 Studd, J W W Blainey J D & Bailey D E. A study of serum protein changes in late pregnancy and identification of the pregnancy zone protein using antigen antibody crossed immunoelectrophoresis. J Obstet Gynaec Brit Comm 77 42, 1970.
29 Thompson, R. P H & Williams, R. Contraception with chlormadinone acetate in woman with previous contraceptive jaundice. Brit Med J 1 152, 1970.
30. Wolf A. V Fuller J B., Goldman, E J & Mahony T D: New refractometric methods for the determination of total proteins in serum and in urine Clin Chem 8 158 1962.
31 Ylöstalo, P Liver function in hepatosis of pregnancy and pre-eclampsia with special reference to modified bromsulphthalein tests. Acta Obstet Gynec Scand 49 Suppl. 4 1970.

Submitted for publication June 16 1972

Pekka Ylöstalo
Department of Obstetrics and Gynaecology
University Hospital
Oulu
Finland

Acta Obstet Gynec Scand 52, 229–234 1973

TRIMETHOPRIM-SULFONAMIDE COMBINATION ADMINISTERED ORALLY AND INTRAVAGINALLY IN THE FIRST TRIMESTER OF PREGNANCY ITS ABSORPTION INTO SERUM AND TRANSFER TO AMNIOTIC FLUID

O Ylikorkala, E. Sjöstedt, P A. Järvinen, R. Tikkanen and T Raines

From the Department of Obstetrics and Gynaecology (Head. Prof P A. Järvinen), University of Oulu, Oulu, and Medical Research Laboratories (Head. R. Tikkanen) (Lääke Oy & Medipolar Oy), Turku, Finland

Abstract. The absorption of trimethoprim-sulfonamide combination into serum and transfer to the amniotic fluid after oral and intravaginal administration was studied on 134 patients undergoing legal abortion. The combination given orally as single dose was absorbed rapidly the peak levels of both trimethoprim and sulfamethoxazole in serum were reached within 4 hours. In amniotic fluid, the peak level of trimethoprim appeared within 14 hours and that of sulfamethoxazole within 18 hours. After single intravaginal application of cream, the absorption of trimethoprim into serum and transfer to the amniotic fluid as smaller although measurable amounts could be demonstrated. After repeated intravaginal application during several days no accumulation of trimethoprim in serum or amniotic fluid could be detected.

Trimethoprim, 2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimide, which is an inhibitor of dihydrofolic acid reductase, has a bacteriostatic effect, like sulfonamides. The effect of trimethoprim and sulfonamides combined is synergistic and bactericidal to most of the pathogenic bacteria (5, 6, 8, 10). Trimethoprim is well absorbed from the alimentary tract. It is excreted almost unchanged by the kidneys, and attains there concentrations which are many times higher than serum concentrations (4, 6, 15, 17). Transfer of trimethoprim to the amniotic fluid has not been studied previously. Nor is it known to what extent trimethoprim applied as vaginal cream is absorbed into the serum and amniotic fluid.

In this investigation the absorption and transfer to the amniotic fluid of an orally administered trimethoprim-sulfamethoxazole combination studied. Absorption of trimethoprim from a vaginal cream containing sulfafurazole and trimethoprim has also been studied.

MATERIAL AND METHODS

The material consisted of 134 patients who underwent legal abortion (in the period between June 1 November 15 1971) at the Department of Obstetrics and Gynaecology University of Oulu. Gestation periods ranged from 8 to 20 weeks, with an average of 11 weeks.

The following preparations were used in the study: A tablet containing 80 mg trimethoprim and 400 mg sulfamethoxazole (Trimosulfa® Lääke Oy). A vaginal cream containing 2% trimethoprim and 10% sulfafurazole in the basic cream of lanolin, paraffin oil, propylene glycol, Tegacide, and methyl- and propyl-p-oxibenzoate, acidified with lactic acid

I the first group of patients single oral dosage of two tablets, containing 160 mg trimethoprim and 800 mg sulfamethoxazole, was given to patients who had been without food for 8 hours. Blood samples were taken by venepuncture from ten patients before, and 2, 4, 6, 8 12, 14, 16, and 18 hours after medication.

An amniotic fluid sample was taken from each of 44 patients by amniotic puncture through the cervical canal after careful [illegible] of vagina either 2, 4, 6, 8 12, 14, 16, or 18 hours after medication. Trimethoprim and total sulfonamide concentrations were determined in serum and amniotic fluid, with the exception of the samples taken 14 16, and 18 hours after the medication, when total sulfonamide concentration was not determined.

In the second group of patients one applicatorful cream (= 5 g) containing about 100 mg trimethoprim and 500 mg sulfafurazole was introduced into the vagina. Blood samples were taken by venipuncture from 10 patients before and 2, 4, 6, 8, and 12 hours after

Table I Concentration of trimethoprim and total sulfonamide (µg/ml) in serum 2–18 hours after a oral single dose of trimethoprim-sulfamethoxazole-combination containing 160 mg trimethoprim (2.5 mg/kg) and 800 mg sulfamethoxazole (12.5 mg/kg)

T = trimethoprim, S = sulfamethoxazole

		2 h		4 h		6 h		8 h		12 h		14 h		16 h		18 h	
Patient	Weight	T	S	T	S	T	S	T	S	T	S	T	S[a]	T	S[a]	T	S[a]
A	56.00	1 09	44 00	1.30	42.30	0.72	33.10	0.65	29.30	0.61	21.30	0.43	—	0.38	—	0.37	—
B	67.00	1 14	48.40	1.24	52.80	0.73	42.40	0.56	35.00	0.35	25.20	0.35	—	0.32	—	0.32	—
C	72.00	1.02	60.00	1.28	56.40	1.37	34.80	0.91	29.20	0.65	19 60	0.63	—	0.50	—	0 4.	—
D	63 00	1.35	40.80	1 90	55.60	1.57	46.40	0.84	36.80	0 48	30.00	0.49	—	0.32	—	0.32	—
E	76.00	1 45	59.20	1.37	50.80	1.30	40.20	0.94	32.40	0 66	22.60	0.37	—	0.31	—	0.23	—
F	72.00	1.85	44 80	1.80	47 60	1.30	46.00	1 05	34 40	1.00	32.40	0.61	—	0.45	—	0.35	—
G	48.00	.20	35.00	2.10	88.40	1 17	78.80	1 14	69.80	0.85	52.80	0.52	—	0.48	—	0.39	—
H	58.00	1.81	1 60	1 66	19.20	1.27	30.40	0.97	36.20	0.36	26.80	0.45	—	0.37	—	0.33	—
I	*63.00*	*0.80*	*54.80*	*1.56*	*48.80*	*1 72*	*36.80*	*1 69*	*30.40*	*1.22*	*21 60*	*0.62*	—	*0.64*	—	*0.38*	—
J	78.00	1.27	63.20	0.91	50.40	0.85	40.20	0.70	35.20	0.51	27 60	0.48	—	0.32	—	0.28	—
Mean value		1 40	47 18	1.51	51.23	1.20	42.91	0.94	36.87	0.67	27.99	0 49		0.41		0.34	
S.E.M ±		0.13	3 85	0.11	5 06	0.10	4.10	0.10	3.57	0 08	2.89	0 03		0.03		0.02	

[a] Not determined.

medication. An amniotic fluid sample was taken from each of 4 patients by the same method as after the oral administration, ., 4 6, 8 or 1 hours after the medication. Blood contaminated samples were rejected. Only trimethoprim levels were determined in the serum and amniotic fluid.

In the third group of patients one applicatorful of cream was inserted into vagina each evening for 7–10 days. Serum samples were taken from 28 patients before treatment and 1.–18 hours after the last application. Amniotic fluid samples were taken from 16 patients after complication of treatment. Umbilical cord blood was obtained from the fetuses of two patients undergoing vaginal hysterotomy gestation age 13th and 16th weeks respectively

In all these samples only trimethoprim levels were determined. Trimethoprim was assayed microbiologically from the samples by an agar-diffusion method using Bacillus pumilus E 601 (SBL) as a test organism. In order to inactivate sulfonamide 5 mg/100 ml p-aminobenzoic acid was added to the medium. The sensitivity limit of the method is ≥ 0.01 µg/ml (6). Total sulfonamide concentration was determined by the method of Bratton & Marshall (3).

RESULTS

The results are presented in Tables I II and III and in Figs. 1 and 2. Table I shows that following oral administration therapeutic levels in serum for both trimethoprim and sulfamethoxazole are reached within 2 hours. Peak levels for both agents occur in 4 hours with a sulfonamide level con centration approximately 35 times higher than that of trimethoprim (trimethoprim 1.51 µg/ml ± 0.11 and total sulfonamide level 51.. µg/ml ± 5 06). The concentrations of both drugs decrease thereafter at the same rate After 18 hours the trimethoprim level is still 0.34 µg/ml ± 0.02. The biological half life of trimethoprim in serum can thus be calculated to be about 11 hours.

Table II shows that following the oral administration average levels for trimethoprim in amniotic fluid after ? 4 and 6 hours are approximately 10 per cent of the corresponding serum levels, rising to 50% at 12 hours and 70% at 14 hours. The peak concentration is at 14 hours (0.37 µg/ml ± 0.04). After 18 hours trimethoprim level in amniotic fluid is still 0 25 µg/ml ± 0 0.. The concentration peak for total sulfonamide in amniotic fluid is reached after 10 hours (7 9 µg/ml ± 3 55) when it is a little over ?0% of the corresponding serum level. Levels after 1 hours were not determined Sulfonamide levels in the amniotic fluid are about 30 times those of trimethoprim.

Table III shows serum levels for trimethoprim ?–1. hours after intra aginal application of trimethoprim-sulfafurazole cream. In three of the 10 patients trimethoprim was detected in serum within . hours. By 4 hours the drug was detectable in 9/10, and by 6 hours in 10/10. Detectable amounts of the drug were still present after 12 hours in seven cases out of ten. Peak level occurred after approximately 6 to 8 hours.

Table II. *Concentration of trimethoprim and total sulfonamide in amniotic fluid (µg/ml) of 44 patients 2–18 hours after a single oral dose of trimethoprim-sulfamethoxazole-combination containing 160 mg trimethoprim and 800 mg sulfamethoxazole*

T trimethoprim, S sulfamethoxazole

	2 h		4 h		6 h		8 h		10 h		12 h		14 h		16 h		18 h	
	T	S	T	S	T	S	T	S	T	S	T	S	T	S*	T	S*	T	S*
	0.15	4.8	0.13	4.4	0.05	1.2	0.25	4.9	0.37	10.1	0.43	6.8	0.42	—	0.11	—	0.31	—
	0.19	2.5	0.15	6.1	0.17	3.6	0.05	1.1	0.30	6.8	0.21	7.2	0.32	—	0.23	—	0.20	—
	0.14	2.4	0.14	4.0	0.16	3.0	0.20	5.6	0.24	5.2	0.17	5.4	0.32	—	0.41	—	0.24	—
	0.10	2.6	0.16	6.4	0.17	6.5	0.39	9.8	0.12	1.6	0.30	5.5	0.25	—	0.33	—	0.23	—
	0.05	0.6	0.12	4.9	0.35	22.9	0.20	16.8	—	—	0.31	12.0	0.34	—	0.36	—	0.25	—
Mean value	0.13	2.58	0.14	5.60	0.16	7.44	0.22	7.64	0.26	7.93	0.28	7.38	0.37	—	0.29	—	0.25	—
S.E.M. ±	0.02	0.40	0.01	0.44	0.03	3.54	0.05	2.39	0.05	3.55	0.04	1.08	0.04	—	0.06	—	0.02	—

* Not determined.

Total sulfonamide levels in serum were not determined.

After a single intravaginal application of trimethoprim-sulfafurazole cream to 42 patients, measurable amounts of trimethoprim could be demonstrated in amniotic fluid as follows: in the samples after hours in two cases out of six (0.01 µg/ml in both), in the samples after 4 hours in none out of nine, in the samples after 6 and 8 hours in two and one out of ten respectively and in the samples after 12 hours in six out of seven. Levels for trimethoprim in the amniotic fluid ranged between 0.01–0.11 µg/ml. Total sulfonamide determinations were not performed.

In the 28 patients who received a 7–10 day course of trimethoprim-sulfafurazole vaginal cream, trimethoprim was demonstrated in the serum of 17 with levels ranging from 0.01 to 0.06 µg/ml. Amniotic puncture was successfully performed in 16 cases. Detectable amounts of trimethoprim were demonstrated in three cases. In two naval blood sample trimethoprim could not be demonstrated.

Fig. 1 shows the serum and amniotic fluid levels for trimethoprim after a single oral or intravaginal administration as a function of time. Fig. 2 shows respective serum and amniotic fluid levels for sulfamethoxazole after a single oral administration.

DISCUSSION

A combination of trimethoprim-sulfamethoxazole oral is effective in the treatment of urinary tract infections (2, 11, 13, 16), in chronic bronchitis (9), and in gonorrhea (7). Trimethoprim alone prevents bacterial growth in most cases, but the degree of synergy with sulfonamide is high and the increase in efficacy approximately 4–8-fold (6, 8). Most studies conclude that the optimum ratio for trimethoprim and sulfonamide combination in an oral preparation is 1 : 5 (Trimosulfa® tabl.) (10).

Trimethoprim prevents the bacterial synthesis of folic acid by inhibiting the action of dihydrofolate reductase. In order to inhibit the corresponding mammalian enzyme, 10 000 to 50 000 times higher trimethoprim concentrations would be needed, according to various reports (12, 14). Because of species differences in the reductase

Table III. *Concentration of trimethoprim in serum (µg/ml) 2–12 hours after single intravaginal application of trimethoprim-sulfafurazole-ointment containing about 100 mg of trimethoprim*

Patient	Weight	2 h	4 h	6 h	8 h	12 h
K	57.00	0.0	0.03	0.03	0.04	0.00
L	64.00	0.0	0.0	0.03	0.02	0.02
M	59.00	0.0	0.04	0.05	0.06	0.06
N	63.00	0.0	0.01	0.02	0.03	0.03
O	57.00	0.0	0.02	0.04	0.04	0.0
P	66.00	0.02	0.04	0.03	0.03	0.03
R	54.00	0.04	0.06	0.04	0.04	0.04
S	63.00	0.0	0.02	0.03	0.03	0.02
T	60.00	0.0	0.02	0.02	0.04	0.03
U	54.00	0.02	0.03	0.05	0.04	0.0
Mean value		0.01	0.01	0.04	0.04	0.02
S.E.M. ±		0.004	0.01	0.003	0.01	0.01

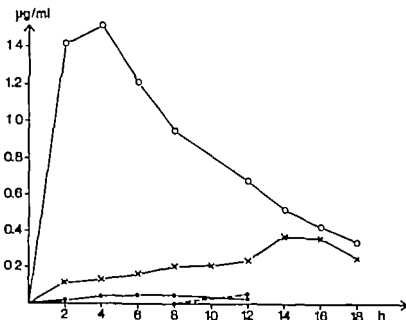


Fig 1 Trimethoprim level in serum o—o and amniotic fluid x—x after a single oral dose of trimethoprim-sulfamethoxazole combination containing 160 mg trimethoprim and 800 mg sulfamethoxazole (see Tables I and II). Trimethoprim level in serum •—• and amniotic fluid • -• after a single intravaginal application of trimethoprim-sulfafurazole cream containing about 100 mg trimethoprim and 00 mg sulfafurazole (see Table III and the text).

enzyme, deficiency in the folic acid metabolism during treatment in man has not been observed (2, 4 14 16) Large doses of trimethoprim sulfonamide combination have been observed to have teratogenic effect (19). However Williams et al. (20) found no difference from the normal ratio of malformations after they had treated urinary tract infections in 120 pregnant women with the combination of trimethoprim and sulfonamide

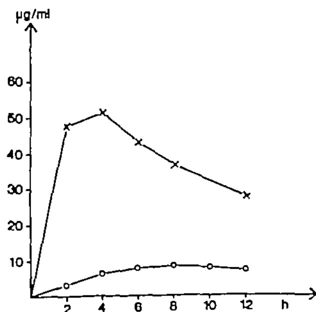


Fig 2 Total sulfonamide level in serum — and amniotic fluid o—o after a single oral dose of trimethoprim-sulfamethoxazole combination containing 160 mg trimethoprim and 800 mg sulfamethoxazole (see Tables I and II).

Preparations containing trimethoprim are not recommended during pregnancy That is why the subjects in this experiment were patients undergoing legal abortion. Folic and metabolism was not investigated, nor were embryos systematically studied since the experiment involved a single dosage only therapy In order to throw light on eventual teratogenic effects, a new series of experiments with long lasting trimethoprim treatment should be undertaken and the embryos submitted to careful histological examination.

In the present study no side-effects following either the oral or intravaginal regimen were observed.

Trimethoprim was absorbed rapidly from the alimentary canal The peak levels occurred within 4 hours, a finding in agreement with previous reports (4 6, 15) The biological half-life of trimethoprim in the study is calculated to be about 11 hours, which also corresponds with the values reported in the literature (6 17). Sulfamethoxazole concentration in the serum was approximately 35 times higher than the trimethoprim concentration which agrees with previous data (4 17) Transfer of trimethoprim via the placenta into the amniotic fluid and blood circulation of foetus has never been studied before in man. The gestational period of the patients in our study ranged from 8 to 20 weeks, the average being 11 weeks. Trimethoprim was transformed to the amniotic fluid rather slowly the peak level not occurring until 14 hours. Trimethoprim is about

31–44% bound to serum proteins. The conjugation may influence the transfer rate.

Only two umbilical cord blood samples were taken in the study. The patients concerned inserted an applicatorful vaginal cream into the vagina each night for at least one week. Trimethoprim could not be demonstrated in either sample. Following oral dosage of the combination, the measured trimethoprim concentrations in the amniotic fluid may reach therapeutic levels (5, 6, 8, 10).

There is no mention in the literature of the use of a combination of trimethoprim and sulfonamide as a vaginal cream, nor about the absorption from this combination into the serum or amniotic field. The absorption rate of trimethoprim into serum observed in the present study was ery slight (Table III) and no cumulation of trimethoprim during the 7–10 days course was found.

After intravaginal application trimethoprim could be demonstrated in only 11 out of 42 successful amniotic fluid samples. In evaluating the amniotic fluid results, the possibility of contamination has to be considered: trimethoprim could be carried into the sample by the needle after even most careful washing of the vagina, and thus produce measurable levels. Probably trimethoprim was to some extent carried through the cervical canal directly into the amniotic fluid, because 12 hours after the application of the cream, the a erage amniotic fluid level for trimethoprim was higher than the verage serum level whereas after oral administration the serum level for trimethoprim was always higher than the amniotic fluid level.

Serum and amniotic fluid levels of trimethoprim after intravaginal application appear too small to ha e any adverse effect on the patient's folic acid metabolism.

Vaginal bacterflora and changes during treatment with a cream containing trimethoprim and sulfafurazole were followed in 31 patients. The results appear promising, and the research will be carried on with a further series of xperiments, in order to clarify the appropriateness of the vaginal cream containing trimethoprim for the therapy of vaginitis.

REFERENCES

1. Akinkugbe, O. O. Lewis, E. A., Montefiore, D. & Okubadejo, O. A.: Trimethoprim and sulphamethoxazole in typhoid. Brit Med J *3* 721, 1968.
2. Allison, M. E. M., Kennedy A. C., McGeachie, J & McDonald, G. A.: Sulphamethoxazole-trimethoprim therapy in urinary tract infection with reference to its haematological effects. Scot Med J *14* 355 1969
3. Bratton, A. C. & Marshall, E. K.: A new coupling component for sulfanilamide determination. J Biol Chem *128* 537 1939
4. Brumfitt, W. Faiers, M. C., Pursell, R. E., Reeves, D. S. & Turnbull, A. R.: Bacteriological, pharmacological and clinical studies with trimethoprim-sulphonamide combinations. Postgrad Med J Suppl *45* 56, 1969
5. Bushby, S. R. M.: Combined antibacterial action in vitro of trimethoprim and sulphonamides. Postgrad Med J Suppl *45* 10, 1969
6. Bushby, S. R. M. & Hitchings, G. H.: Trimethoprim, sulphonamide potentiator. Brit J Pharmacol *33* 72, 1968.
7. Csonka, G. J.: Therapeutic trial of trimethoprim as potentiator of sulphonamides in gonorrhoea. Br J Vener Dis *43* 161, 1967
8. Darrel, J. H. Garrod, L. P. & Waterworth, P. M.: Trimethoprim: laboratory and clinical studies. J Clin Pathol *21* 202, 1968.
9. Drew C. D. M., Hughes, D. T. D. Fowle, A. S. E. & Cassell, M. A.: Effective treatment of chronic bronchitis with short-term trimethoprim and sulphamethoxazole. Proc. 5th International Congress of Chemotherapy Vienna, June 25 July 2, 1967 Suppl.
10. Garrod, L. P.: The possible scope of trimethoprim-sulphonamide treatment. Postgrad Med J Suppl *45* 52, 1969
11. Grüneberg, R. N. & Kolbe, R.: Trimethoprim in the treatment of urinary infections in hospital. Brit Med J *1* 545 1969.
12. Hitchings, G. H.: Species differences among dihydrofolate reductases as basis for chemotherapy. Postgrad Med J Suppl. *45* 7 1969
13. Hoigné, R., Müller U. & Schneider H. R.: Bactrim Roche, ein Kombinationspräparat von Sulfamethoxazol und Trimethoprim. Schweiz Med Wschr *99* 1511, 1969
14. Kahn, S. B. Fein, S. A. & Brodsky I.: Effects of trimethoprim on folate metabolism in man. Clin Pharmacol Ther *9* 550, 1968.
15. Kaplan, S. A., Weinfeld, R. E., Cotler S. Abruzzo, C. W. & Alexander K.: Pharmacokinetic profile of trimethoprim in dogs and man. J Pharm Sci. *59* 358, 1970
16. Kosmaris, A. & Forsström, J.: Observations on the use of combination of trimethoprim and sulphamethoxazol in the treatment of urinary tract infections. Duodecim *86* 1104, 1970.
17. Schwartz, D. E. & Rieder J.: Pharmacokinetics of sulphamethoxazole + trimethoprim in man and their distribution in the rat. Chemotherapy *15* 337 1970.

18. Schwartz, D E & Ziegler W H. Assay and pharmacokinetics of trimethoprim in man and animals. Postgrad Med J Suppl. 45 3., 1969
19 Udall, V Toxicology of sulphonamide-trimethoprim combination. Postgrad Med J Suppl. 45 42, 1969
20. Williams, J D Brumfitt, W Condie A P & Reeves, D S The treatment of bacteruria in pregnant women with sulphamethoxazol and trimethoprim. Postgrad Med J Suppl. 45 71 1969

Submitted for publication Aug 3 1972

O. Ylikorkala
Department of Obstetrics and Gynaecology
University Clinic
Oulu
Finland

Acta Obstet Gynec Scand 52 235–243 1973

ESTIMATION OF GESTOSIS OF PREGNANCY (EPH-GESTOSIS)

Leo Tervilä, Claus Goecke and Sakari Timonen

From Department I of Obstetrics and Gynecology Helsinki University Central Hospital, Helsinki, Finland (Head: Prof S. Timonen M.D) and the University Womens' Clinic (Head: Prof Dr H Schwalm), Würzburg, Germany

Abstract Systolic and diastolic blood pressure, urinary protein and amount of edema were compared with factors reflecting the prognosis and condition of the newborn in series of 4 404 patients with EPH-gestosis, collected from the Helsinki University Central Hospital in 1963–1969 and the University Women Clinic, Würzburg, in 1960–1969 No significant association was observed between slight and moderate edema and the prognosis of the newborn. The systolic blood pressure gave more significant information than the diastolic blood pressure. The amount of urinary protein showed the most consistent correlation with an impaired prognosis. The systolic blood pressure and proteinuria were thus the most significant indicators An index for estimation of the prognosis in EPH-gestosis was constructed on this basis. The purpose of this index is to provide means of assessing the severity of the condition in the initial stage of treatment by exact use of simple observations The index defines the risk of perinatal mortality prematurity asphyxia and smallness for dates in the individual case.

In recent years many investigators have posed the question as t whether it is possible—when a gravida with gestosis is admitted to hospital—to estimate her and her baby's prognosis on the basis of the three classic signs: edema (E) proteinuria (P), and hypertension (H) (1 2, 4 7) The goal has been to determine the correlation between each symptom at various levels of severity and the condition of the baby The increasing use of automatic data processing in medicine necessitates the quantitation and numbering of clinical observations An index of this kind may also be helpful when significant information concerning the patient history and laboratory results are not available

The need for simple visual model has been particularly emphasized, so that the situation can be evaluated immediately on admission of the patient (5) Furthermore an index ought to enable a comparison of the results from different hospitals or different countries. Hence it is desirable that subjective evaluations are avoided as far as possible.

The construction of an index requires very careful analysis of the material by statistical methods Moreover the material must be large and unbiassed.

The purpose of this investigation is to construct an index as described above. The material used was collected from two different countries.

MATERIAL AND METHODS

The material consists of 2 077 consecutive cases of EPH-gestosis from the Universitäts-Frauenklinik, Würzburg from 1960–1969 and 2 327 cases from the Helsinki University Central Hospital from 1963–1969 which makes total of 4 404 cases The patients were in the third trimester of pregnancy All of them were examined for the classic symptoms of gestosis. The criteria for this series were systolic blood pressure over 140 mmHg, diastolic blood pressure over 85 mmHg, positive Esbach test for proteinuria and the presence of significant edema. Patients showing some other disease such as diabetes or rhesus-incompatibility are omitted. Besides, no attention was paid to the previous course of pregnancy The series thus includes women with essential hypertension and chronic renal disease Moreover, data on the babies were collected. Perinatal mortality was defined as death during pregnancy or delivery or within seven days after birth The criterion of asphyxia was one-minute Apgar score of 0–6. Babies with birth weight under 2 500 g were regarded as premature The percentage of small-for dates babies as obtained from the birth-weight figures and duration of gestation taking into account that in the general population 10% of the newborn are small for dates according to probability table previously made (9) Other factors considered, are the length and weight of the newborn baby weight of the placenta and duration of pregnancy As indicated by the frequencies of

Table I *Distribution of the cases*

	No. of cases	Eclampsia (%)	Perinatal mortality (%)
Helsinki	2 327	0.9	5.1
Würzburg	2 077	3.8	10.0

eclampsia and by the perinatal mortality rate, the Würzburg material clearly contained more severe cases than did that of Helsinki (Table 1)

RESULTS

The systolic blood pressure was correlated to perinatal mortality and frequency of babies Apgar scored 0–6 babies with a birth weight below 2 500 g and babies small for date (Fig. 1). As Fig. 1 indicates, the frequency of babies with low Apgar scores as well as frequency of small for dates babies rose more abruptly when the systolic blood pressure exceeded 175 mmHg. This parameter thus seems to be the most sensitive indicator of an abnormal situation

Similar changes were observed in correlating the length of the baby the duration of pregnancy (Fig. 2) the weight of the baby and placenta (Fig. 3). The relative weight of the placenta rose from about 18.5% to 20% while the blood pressure rose over 175 mmHg. The fetal weight decreased more than the weight of the placenta (Fig. 3) Similar associations with raised diastolic blood pressure were shown but were less pronounced, the critical value being 115 mmHg. The correlations with the diastolic blood pressure are shown in Figs. 4 5 and 6.

The curves expressing the correlations between proteinuria and the four main factors in the babies (Fig. 7) are more linear than those shown in Figs. 1 and 4 There is an increase in complications when proteinuria rises over 2‰. The curve expressing the ratio of placental weight to the weight of the newborn showed a marked increase when proteinuria exceeds 3.5‰ Esbach (Fig. 8).

The correlations obtained from the blood pressure and proteinuria are very much alike in the Helsinki and Würzburg series. The results were pooled therefore On the contrary the effect of edema on fetal complications should be presented separately since this sign of EPH gestosis was differently assessed in the two hospitals (Fig. 9). In the Helsinki series the prognosis of the newborn improved with a shift from non-edematous to slight and pronounced edema but in the Würzburg series gross edema was associated with a worse prognosis. Since severe gestosis was less frequent in Helsinki than in Würzburg, gross edema was obviously rare in the Helsinki patients. The severity of edema in the Würzburg series is reflected by a marked reduction in weight of the foetus as compared to the placenta and a consequent rise of the placental weight ratio (Fig 10) This is in contrast to the results in the Helsinki series.

Using these results, correlation coefficients were calculated for the relationship between the dif

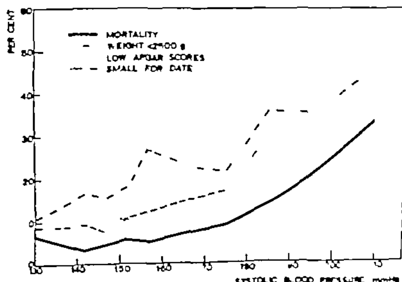


Fig. 1 Systolic blood pressure correlated with the indicators of the prognosis of the newborn.

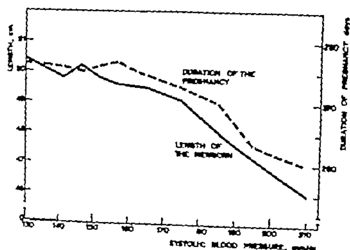


Fig. 2. Systolic blood pressure correlated with duration of pregnancy and length of the newborn.

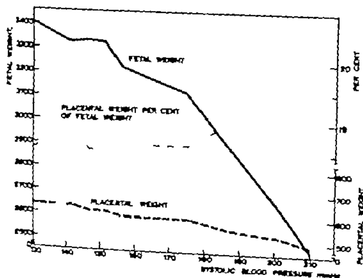


Fig. 3. Systolic blood pressure correlated with fetal and placental weight.

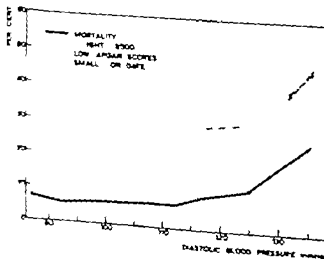


Fig. 4. Diastolic blood pressure correlated with the indicators of the prognosis of the child.

Table I *Distribution of the cases*

	No. of cases	Eclampsia (%)	Perinatal mortality (%)
Helsinki	2 327	0.9	5 1
Würzburg	2 077	3.8	10.0

eclampsia and by the perinatal mortality rate the Würzburg material clearly contained more severe cases than did that of Helsinki (Table I).

RESULTS

The systolic blood pressure was correlated to perinatal mortality and frequency of babies Apgar scored 0–6 babies with a birth weight below 2 500 g and babies small for date (Fig. 1) As Fig. 1 indicates, the frequency of babies with low Apgar scores as well as frequency of small for dates babies rose more abruptly when the systolic blood pressure exceeded 175 mmHg. This parameter thus seems to be the most sensitive indicator of an abnormal situation

Similar changes were observed in correlating the length of the baby the duration of pregnancy (Fig. 2) the weight of the baby and placenta (Fig. 3). The relative weight of the placenta rose from about 18.5% to 20% while the blood pressure rose over 175 mmHg. The fetal weight decreased more than the weight of the placenta (Fig. 3) Similar associations with raised diastolic blood pressure were shown but were less pronounced the critical value being 115 mmHg. The correlations with the diastolic blood pressure are shown in Figs. 4 5 and 6

The curves expressing the correlations between proteinuria and the four main factors in the babies (Fig. 7) are more linear than those shown in Figs. 1 and 4 There is an increase in complications when proteinuria rises over 2‰. The curve expressing the ratio of placental weight to the weight of the newborn showed a marked increase when proteinuria exceeds 3.5‰ Esbach (Fig. 8).

The correlations obtained from the blood pressure and proteinuria are very much alike in the Helsinki and Würzburg series. The results were pooled therefore. On the contrary the effect of edema on fetal complications should be presented separately since this sign of EPH-gestosis was differently assessed in the two hospitals (Fig. 9). In the Helsinki series the prognosis of the newborn improved with a shift from non-edematous to slight and pronounced edema, but in the Würzburg series gross edema was associated with a worse prognosis. Since severe gestosis was less frequent in Helsinki than in Würzburg gross edema was obviously rare in the Helsinki patients. The severity of edema in the Würzburg series is reflected by a marked reduction in weight of the foetus as compared to the placenta and a consequent rise of the placental weight ratio (Fig 10) This is in contrast to the results in the Helsinki series.

Using these results, correlation coefficients were calculated for the relationship between the dif

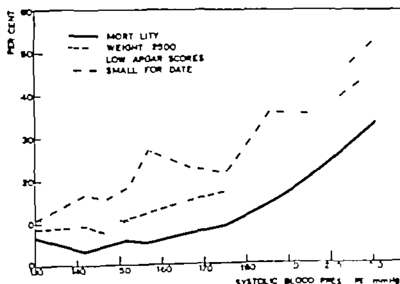


Fig 1 Systolic blood pressure correlated with the indicators of the prognosis of the newborn.

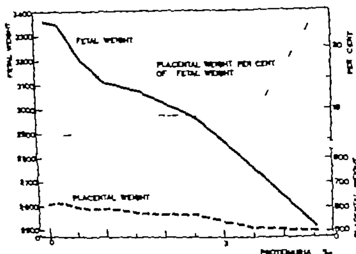


Fig. 8. Excretion of urinary proteins correlated with fetal and placental weight.

ferent symptoms of EPH-gestosis and the factors reflecting the condition of the newborn (Table II). Edema was found to be negatively correlated with smallness for dates and prematurity of the baby. However the correlations of edema with the other factors were not significant, partly because the number of patients with pronounced edema was still too small.

The elevated blood pressure and the increase of protein excretion showed highly significant positive correlations with the condition of the newborn. Proteinuria proved to be the most significant factor. In order to test the significance of the systolic and diastolic blood pressures, partial correlations with the variables for the condition of the newborn were calculated by eliminating in turn the effect of urinary protein as well as systolic and diastolic blood pressure (Table III).

The systolic blood pressure was found to be the

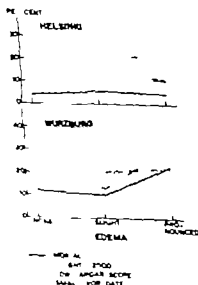


Fig. 9. The degree of edema of the mother correlated with the outcome of the pregnancy of the newborn.

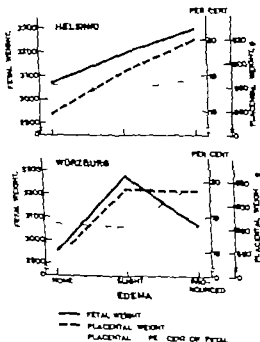


Fig. 10. The degree of maternal edema correlated with fetal and placental weight.

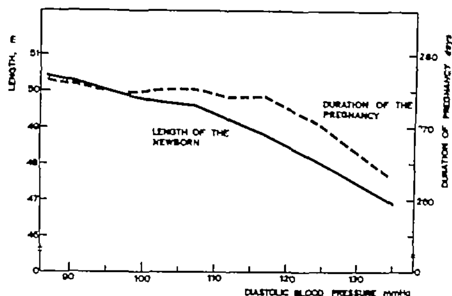


Fig 5 Diastolic blood pressure cor related with duratio of pregnancy and length of the newborn.

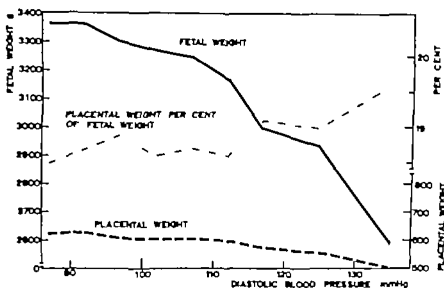
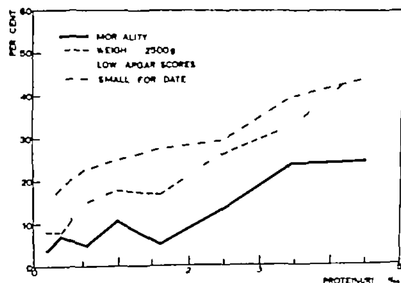


Fig 6 Diastolic blood pressure cor related with fetal and placental weight.



F g 7 Excretion f rinary proteins cor related w th the indicators of the prognosis of the ne born.

Table V *Proposed gestosis index*

Proteinuria in ‰	Systolic blood pressure, mmHg			
	–149	169	189	190
0	0	1	2	9
0.1–0.7	0	3	5	14
0.8–2.9	4	5	10	18
3.0–	7	14	20	27

almost linear positive correlation was obtained (Fig. 11). The same is true for the other correlations discussed before. With an increasing index, both the placenta and foetus are smaller especially the latter. This results in an increase in the foetal placental weight ratio (Fig. 12).

DISCUSSION

Distinction between mild and severe EPH-gestosis

Of the curves illustrating the effect of the various signs of gestosis on the prognosis of the baby that related to proteinuria (Fig. 7) reveals a steady impairment of prognosis with a rising level of urinary protein. The curve is almost linear. By contrast, the curves for the two blood pressures (Figs. 1 and 4) show an initially slow and later more abrupt rise. This abrupt impairment

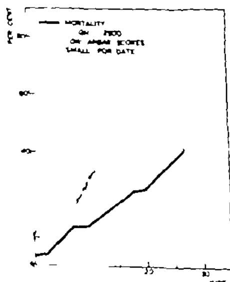


Fig. 11. The proposed gestosis index correlated with the indicators of fetal prognosis.

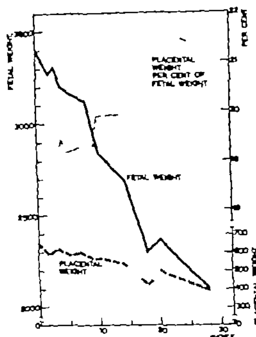


Fig. 12. The proposed gestosis index correlated with fetal and placental weight.

of prognosis starts at about 175 mmHg systolic and 115 mmHg diastolic blood pressure. These figures may thus be considered as borderline values for severe gestosis. The rise moreover appears to start more or less at the same level in the two curves, indicating a similar prognosis. If the borderline value for severe proteinuria is chosen to correspond with the same impairment of the prognosis as in hypertension, a figure of 2‰ urinary protein is obtained.

On the basis of our index a score of 10, for instance, could be taken as the borderline value between mild and severe toxaemia. The graphs show that a patient with a score of 10 will be delivered of a baby who measures on average 48 cm and has a birth weight of 2 880 g, a 32% risk of being small for dates and a perinatal mortality risk of 15%.

The lower limit of mild EPH-gestosis

It is much more difficult to determine the level where mild gestosis starts. Concerning proteinuria, a slight impairment of the score for the baby was observed as soon as proteinuria became evident. It is even possible that the rise of the curve illustrating this correlation starts before

Table II *Correlation coefficients for the relationship between various signs of gestosis factors reflecting the condition of the newborn*

	Mortality	Prematurity	Low Apgar scores	Small for dates	Duration of pregnancy
Edema (Helsinki)	*+0.01*	*−0.04*	*−0.02*	*−0.07*	*−0.02*
Edema (Würzburg)	−0.02	−0.09	—	−0.09	−0.08
Proteinuria (pooled)	+0.198	+0.251	+0.112	+0.192	+0.159
Syst. BP (pooled)	+0.155	+0.228	+0.097	+0.176	+0.144
Diast. BP (pooled)	+0.093	+0.186	+0.046	+0.155	+0.107

Levels of significance 0.03 – $p<0.05$ 0.04 – $p<0.01$ 0.05 – $p<0.001$

factor which significantly influences the prognosis of the baby after the elimination of the effect of the other symptoms. The correlation of the diastolic blood pressure with the frequency of prematurity and smallness for dates is less significant for use in clinical practice. Considering the slight effect of edema and the diastolic blood pressure on the prognosis of the baby these variables were neglected. The gain in simplicity of the scoring was considerable and the effect of subjective factors inherent in the estimation of edema and diastolic blood pressure was thus eliminated

Then an entire approximate estimate was made. We decided that the prognosis of the baby was best enumerated by a score in which 50% was allotted to neonatal mortality The remaining 50% was shared between prematurity low Apgar scores and smallness for dates. Consequently neonatal death was given a score of 50 and the other three factors were given scores of 17 each. The mean values for the scores for the individual case were calculated and plotted against proteinuria and systolic blood pressure of four degrees each Thus Table IV was obtained presenting the figures as mean values. As to the variance protein accounted for about 60% and systolic blood pressure for about 35% Although the combined effect was for only 5% we decided to make a two-dimensional table since the highest mean scores were obtained in groups with a small number of cases. Consequently their share in the variance is slight compared to the total material The scores for these groups would have been too low if the two symptoms had been rated separately

When the mean values were compared by the [illegible] the difference between a minimum of two ste[illegible]e table was found to be highly significant [illegible]0.001) Adjacent steps in the horizontal and vertical directions did not always differ significantly from each other

The figures were then reduced and rounded off Table V was thus obtained in which 0–27 express the relative prognostic significance of the symptom group in each category When the index was adjusted to the factors used as prognostic indicators in Figs 1 and 5 a very clear and

Table III *Partial correlation coefficients between some signs of severity of gestosis and systolic and diastolic blood pressure*

	Mortality	Prematurity	Low Apgar scores	Small for dates
Systolic BP	0.11	0.13	0.07	0.09
Diastolic BP	0.00	0.07	0.01	0.07

Significance levels for the correlation coefficients 0.03 $p<0.05$ 0.04, $p<0.01$ 0.05, $p<0.001$

Table IV *Mean scores and number of cases in the various groups*

Proteinuria in ‰	Systolic blood pressure, mmHg 149	150–169	170–18[illegible]	1[illegible]–
0	6.10 672	7.40 901	8.45 3 1	17.86 59
0.1–0.7	6.58 338	9.71 464	13.09 172	4.81 47
0.8–2.9	11.40 368	12.66 407	19.06 146	30.00 49
3.0–	15.67 101	4.90 153	32.36 107	41.53 92

Results of two-way variance analysis Effect of proteinuria $F=112.27$ p 0.1 Effect of syst. blood pressure $F=65.60$ p 0.1 Interaction $F=3.79$ p 0.1

9 Timonen, S., Uotila, U., Vara, P., Kauranto, P. & Lokki, O. Forensic medical estimation of the date of conception, intrauterine growth charts. Researches performed by the National Board of Health 5. Helsinki, 1969

Submitted for publication Aug. 6, 1972

Leo Tervilä
Department of Obstetrics and Gynaecology
Helsinki University Central Hospital
Helsinki
Finland

pathological findings are recorded by the rather inaccurate Esbach reaction. Investigation of the so-called microproteins would obviously bring additional information.

With regard to the blood pressure, the frequency of babies small-for-dates increased at 140 mmHg systolic and 90 mmHg diastolic pressure. The other factors only started to rise with hypertensions of 160 mmHg and 105 mmHg. The exact level is difficult to define due to the low rise of the curve It should be taken into consideration that in this series mortality was higher than normal from the outset no normalities were included The above mentioned figures appear to be very high as judged by clinical experience, and the necessity should be to keep the level demanding examination and admission to hospital as low as possible It seems useful to follow previous rules according to which values over 140 mmHg systolic and 90 mmHg diastolic blood pressure were considered pathological.

Edema

The statistical analysis showed that edema was not significantly associated with changing prognosis of the baby An increase in edema even tended to be related to improved prognosis. This observation also made previously (6 8) facilitates decisively objective evaluation of the symptoms of gestosis The comparison of the frequency of gross edema in the two series from different countries clearly revealed a striking discrepancy between the estimates. This discrepancy may be attributed to the lack of a method of determination suited for clinical routine. The techniques measuring the total body water using deuterium or tritium are only suitable for scientific investigations. The antipyridine method, previously much employed has proved to be inaccurate and sometimes unpleasant for the patient.

The abandonment of edema as a prognostic criterion of gestosis does not mean that this manifestation should be ignored. The relative weight increase of the placenta associated with aggravation of edema undoubtedly shows that something significant will happen.

The reduced gestation time perhaps affords a sufficient explanation of this observation. Edema may be the first sign of impending gestosis, thus indicating that the patient belongs to a risk group needing intensive observation

Blood pressure

When the prognosis was assessed on the basis of proteinuria and the systolic blood pressure the diastolic blood pressure gave no significant additional information as shown by statistical analysis. The systolic blood pressure gave more additional information when proteinuria and the diastolic blood pressure were used as a basis. The conclusion is that greater attention should be paid to the systolic than to the diastolic blood pressure. Till recently a contrary view was strongly maintained (3) It should be kept in mind, however that our series does not allow any etiological analysis of different sub-groups.

The index

When the index presented in this paper is compared with that previously published (1) which was based on four variables and thus required summing up of partial values, it is obvious, that the omission of the diastolic blood pressure and edema in any event simplifies calculation and at the same time makes it possible to estimate the prognosis at a glance It is a matter of judgement how far one wishes to carry the simplification of the mathematically exact index shown in Table 16 The 13 variables are readily reduced to 10 for instance but this would be a compromise with exactitude

REFERENCES

1 Goecke C., Vorschlag einer Stadien-Einteilung der Gestose Zbl Gynäk 87 1439 1965
2. Krauss, A., Schlegel, L & Canzler E. Korrelation zwischen Gestosenote und kindlichem Schicksal Gynaecologia (Basel) 166 455 1968
3 Mahran, M A critique on the gestosis index. Gestosis Index (ed. E. T Rippmann) Sandoz Ltd Basle 1971
4 Pankamaa, P Jarvinen, P A & Kinnunen, O Classification of the toxaemias of pregnancy J Obstet Gynaec Brit Emp 64 857 1957
5 Rippmann, E. Th Di Einteilung der Schwangerschaftsspätgestosen Gynaecologia (Basel) 164 15 1967
6 Tervilä, L. & Krokfors, E Correlation of nature of toxaemia of pregnancy to perinatal mortality and underweight of newborn Ann Chir Gynaec Fenn 55 109 1966.
7 Theobald, G W The significance of name Irish J Med Science 51 196
8 Thomson, A M Hytten, F E & Billewicz W Z. The epidemiology of oedema during pregnancy J Obstet Gynaec Brit Comm 74 1 1967

Acta Obstet Gynec Scand 52 245–248 1973

THE COURSE AND OUTCOME OF PREGNANCY IN WOMEN WITH EPILEPSY

Tor Bjerkedal and Sami L. Bahna[1]

From the Institute of Hygiene and Social Medicine (Head: Prof Tor Bjerkedal), University of Bergen, Norway

Abstract Information derived from the compulsory national scheme of medical registration of births in Norway has been utilized in study of the course and outcome of pregnancy in women with epilepsy The series comprises 371 pregnancies in women with epilepsy and, as controls, 112 530 pregnancies in women reporting no disease before or during the pregnancy Comparisons indicate that women ith epilepsy experience an excess of complications during pregnancy and labour and that their babies are more frequently born prematurely and of low birth weight, and moreover have an excess of congenital malformations and higher perinatal and neonatal mortality rates. Epileptics, undoubtedly constitute high risk group in need of special attention during pregnancy and special care during labour A proper interpretation of the findings in terms of causation, however, will call for additional data obtainable only through especially designed inquiries

A national scheme of medical registration of births was introduced in Norway in 1967 (8) Through this scheme information on pregnancies, labours, and newborn babies on a total population basis is made a ailable for central processing and analysis (2)

Of the rious research programmes started, one focuses on factors affecting the course and outcome of pregnancy Factors of particular interest are diseases of the mother One such disease epilepsy is the subject of the present paper

Pregnancy in epileptics has been the concern of many investigators over the years (1 3 4 7 9 10) I the main, interest has been focused on the effect of pregnancy on the epileptic state Only to lesser extent have the studies dealt with the course and outcome of pregnancy in epileptics A review of the literature on this subject till 1932 (1) summarizes the available information, drawing the conclusion that epilepsy apparently does not represent a special risk. This view has later been substantiated (7). On the other hand, evidence to the contrary has also been reported (9).

MATERIAL AND METHODS

The national scheme of medical registration of births which was introduced in Norway in 1967 by the National Health Services is compulsory and calls for information on the mother's health before and during pregnancy as ell as information pertaining to the delivery itself and the condition of the baby Registration comprises all births of 16 weeks and more of gestation. For details on the registration scheme and the information provided, the reader is referred to report which has been issued by and is obtainable through, the Institute (2).

The present series of pregnancies in epileptic women is derived from the registrations during the years 1967 and 1968 Out of 134 348 pregnancies registered in Norway during these two years, there were 371 in women reporting history of epilepsy either before or during the pregnancy The course and outcome of these pregnancies have been compared ith 112 530 pregnancies in women reporting no disease before or during the pregnancy I the comparison of complications occurring during pregnancy omen reporting no disease before pregnancy only altogether 125 423, constitute the controls.

The variables hich are studied include complications during pregnancy conduct of labour gestation period, birth weight, frequency of malformation, disease or birth injury stillbirths, and infant mortality rate. The study of infant mortality rates is made possible through the linkage of the material of the Medical Birth Registry to that of the official registration of deaths made available by the Central Bureau of Statistics of Norway

In the evaluation of differences observed between women with epilepsy and the controls, the chi-square test with 1 degree of freedom has been applied to frequencies, and the one-sided *t*-test ith (n_1 n_2 2) degrees of freedom to arithmetic means. Differences have been considered statistically significant in cases here *p* is less than 0.05

[1] Research fellow on leave from the High Institute of Public Health, University of Alexandria, Egypt

Table III. *Mortality rates for births in women with epilepsy and in women with no recorded disease*

Recorded disease of mother	Total births	Live births	Mortality rates (per 1000)*				
			Stillbirth	Perinatal	Neonatal	Postneonatal	Infant
Epilepsy	378	375	5.3 (2)	31.8 (12)	29.3 (11)	5.3 (2)	34.6 (13)
None	113 311	112 328	7.8 (883)	14.6 (1657)	8.0 (898)	3.4 (382)	11.4 (1280)
Statistical decision			N.S.	$p<0.005$	$p<0.001$	N.S.	$p<0.001$

* Foetal deaths of less than 28 weeks of gestation are excluded in calculations of stillbirth and perinatal mortality rates. Figures in parentheses are the actual numbers of deaths.

mation, which ere to be expected on the basis of the frequency of these malformations among the babies of the controls.

A comparison of mortality in the two groups of births is presented in Table III. It may be seen that the main difference in mortality is experienced in the neonatal period. In this period the death rate among babies of women with epilepsy is more than three times that for babies of the controls. Adjusting the neonatal mortality rates by birth weight considerably reduced the difference leaving, however a rate that is about 70% higher for the babies of the epileptics than for those of the controls.

Information on gestation period and birth weight of *live births* is given in Table IV. The percentages of prematurely-born infants and of infants of low birth weight were significantly higher in epileptic mothers. The mean birth weight of infants of epileptic mothers was 113.2 grams less than in the controls, difference which is highly significant. Exclusion of infants of multiple pregnancy resulted in a reduction of the difference to 98.6 grams which is still statistically significant. Adjusting the mean birth weight by birth order reduced the difference by 22.␣% and adjustment by gestation period reduced it by 14 %.

Hypoxia at birth as also significantly more frequent among the babies of women with epilepsy (1.9%) than in those of the controls (0.7)

DISCUSSION

The present findings indicate that women with epilepsy have higher risk of complications during pregnancy that induction of labour as well as interventions during labour are more often called for that their deliveries are more often complicated, and that their babies have a lower birth weight, a higher frequency of congenital malformations and higher perinatal and neonatal mortality rates.

Of special interest is the observation of a higher frequency of congenital malformations in births of epileptic mothers, particularly of cleft lip and/or palate and urogenital malformation. This finding is contrary to the general conclusion of Hed-

Table IV. *Gestation period and birth weight for live births of mothers with epilepsy and of mothers with no recorded disease*

	Recorded disease of mother		Statistical decision
	Epilepsy	None	
Total number of live births	375	112 328	
Number with recorded gestation period	359	108 622	
Mean gestation period in weeks	39.9	39.9	
Prematurely-born infants (gestation period less than 37 eeks)	8.9	5.0	p 0.01
Number ith recorded birth weight	375	112 000	
Infants of low birth weight (2 500 g or less)	7.4	3.7	p 0.001
Mean birth weight (g)	3 383.5 (diff	3 496.7 113.2)	p 0.001
Birth-order-adjusted mean birth weight	3 401.7 (diff	3 489.8 88.1)	
Gestation-period-adjusted mean birth weight	3 383.5 (diff	3 480.5 97.1)	

Frequency of complications during pregnancy in women with epilepsy compared with women with ɴ ɾded disease before pregnancy

Recorded disease	Total pregnancies	Hyperemesis gravidarum No.		Vaginal haemorrhage No		Toxaemia of pregnancy No.	
Epilepsy	371	5	1.3	19	5 1	28	7.5
None before pregnancy	1_5 423	1 041	0 8	775	2..	5 856	4 7
Statistical decision		N.S.		$p<0.001$		$p<0.01$	

In the comparison of certain variables the direct method or the indirect method of adjustment has been applied. Information on the total birth population of 1967 and 1968 has been used as the standard.

FINDINGS

Pregnancy and Labour

In women with epilepsy multiple birth was observed in 7 pregnancies, i.e. in 1 89% a figure which is significantly higher than in the controls (0 87%).

The frequency of various complications during pregnancy in women with epilepsy and in women reporting no disease before pregnancy is presented in Table I All the main complications namely hyperemesis, vaginal haemorrhage and toxaemia, were more frequent in women with epilepsy Differences with respect to haemorrhage and toxaemia are statistically significant.

Significant differences were also observed with respect to induction of labour 20 2% vs. 9 1% in controls, and complications during labour 19 4% vs. 9 6% in the controls.

A comparison of frequencies of interventions during labour in the two groups is presented in Table II All main types of intervention were significantly more frequent in women with epilepsy in which intervention was recorded for a total of 17 7% of births, compared to only 7 7 in controls.

Births

Births of epileptics were found to be comparable to those of controls with respect to percentage of males (51 3 vs. 51.5) and average age of the mother (26 0 vs. 27 0). Birth-order distributions, however were different first-order births were 53 2% in epileptics vs. 37 0% in controls.

Births with malformation disease or birth injury in general were significantly more frequent in women with epilepsy (9 3%) than in the controls (3 9) Regarding congenital malformation in particular the percentage in births of epileptic mothers was 4.5 twice as much as the .. in the controls. Of the various types of malformation, cleft lip and/or palate and urogenital malformation occurred especially in the births of the epileptic mothers. Each was observed in 4 births, compared with the figures of only 0 6 for cleft lip and/or palate and 0 8 for urogenital malfor

Table II *Frequency of interventions for births in women with epilepsy and in women with no recorded disease*

Recorded disease of mother	Total births	Births with intervention No.		Types of intervention: Rupture of membranes No.	%	Forceps or vacuum ext No		Caesarean section N		Other No	
Epilepsy	378	67	17 7	15	4 0	4	6 3	12	3 2	30	7 9
None	113 511	8 709	7 7	1 635	1 4	2 693	2 4	1 62	1 1	4 369	3 8
Statistical decision		$p<0.001$		$p<0.001$		$p<0.001$		$p<0.001$		$p<0.005$	

Acta Obstet Gynec Scand 52. 249–251 1973

ULTRASONIC FETAL CEPHALOMETRY IN PRE ECLAMPSIA

Per Bergsjö and Christian Brodtkorb

From the Department of Obstetrics and Gynecology (Head, Professor Oddmund Koller), University of Bergen School of Medicine, Bergen, Norway

Abstract. Fetal cephalometry with pulsed ultrasound (A and B scan) has been performed in 35 patients with pre-eclampsia. A classification of fetal growth retardation was based on standard tables for newborn infants. The biparietal diameters were generally small in growth-retarded fetuses, both in relation to fetuses of normal weight in pre-eclamptic mothers, and in relation to the average measurements in a previously compiled group of normal pregnancies.

The fetal biparietal diameter can be measured directly by pulsed ultrasound (4). This technique has been used to compile curves of normal fetal skull growth (2, 6, 9). Such growth curves, from different medical centers, are similar in shape, but average values differ slightly. The growth variance is so small that the biparietal diameter is helpful in the assessment of gestational age in cases of uncertain menstrual dates (1, 2, 8). Serial measurements in the individual case give information about the growth rate of the skull (3, 5, 8). Willock et al. (8) could predict the birth of a dysmature or a non-dysmature baby both with about 70 per cent accuracy. An even better prediction rate was reported by Campbell & Dewhurst (3).

Since pre-eclampsia may be complicated by fetal growth retardation, ultrasonic cephalometry should presumably be useful in such cases. The published results in this condition have, however, been equivocal. Campbell & Dewhurst (3) found that the biparietal growth was retarded in about 50 per cent of their cases, while Jouppila et al. (5) did not find any apparent deviation from normal growth in 22 cases. This discrepancy has prompted us to present our own experience in pre-eclamptic patients during a one-year period. A previously published normal biparietal growth curve, which is similar in shape to those mentioned above, is used as reference (1).

MATERIAL AND METHODS

Patients. The material consists of 35 women with pre-eclampsia, treated at the Department of Obstetrics, Haukeland Hospital. The average stay in the hospital before spontaneous labor or induced birth was between 4 and 6 weeks. There were no cases of diabetes in this series. None of the patients developed eclampsia. The gestational age was known with reasonable certainty in all the cases. Termination of pregnancy was as follows:

Spontaneous labor: 16 cases, 2 of which followed unsuccessful oxytocin induction.

Oxytocin induction: 13 cases, of which 3 were terminated by cesarean section.

Cesarean section: 5 cases.

Rupture of membranes: 1 case (dead fetus).

Classification of the fetuses. The fetuses were classified retrospectively in degrees of dysmaturity according to the tables of birth weight and gestational age compiled by Lubchenko et al. (7): (*a*) those above the 25th percentile (17 cases), (*b*) those between the 25th and the 10th percentiles (18 cases), and (*c*) those below the 10th percentile (10 cases).

Preliminary unpublished data from Norway suggest that the percentile limits for Norwegian children will be higher than those of Lubchenko et al. Therefore it is fair to assume that the majority of children both in groups *b* and *c* were truly growth-retarded.

Biparietal diameter measurements. These were performed by pulsed ultrasound, with an apparatus made by Kretz-Technik, Zipf, Austria (Series 4100 MO). A two-dimensional B-scan was used for orientation, while the actual measurement was done using the one-dimensional A-scan beam. For detailed technical description and illustrations the reader is referred to other sources (4, 8). Altogether 64 measurements were performed in 35 patients, up to in the individual case. The time interval from the last measurement to delivery was 5.93 days on the average. In 26 cases delivery took place within 7 days after the last measurement. Therefore the last point for each patient in Fig. 1 indicates the approximate gestational age at delivery.

RESULTS

The results are presented in Fig. 1. In group *a*, comprising the 17 children with normal birth weights for their gestational age, and marked with

berg et al. (5) who found no significant relation between diseases of the mother and congenital malformations. The finding regarding cleft lip and/or palate is in agreement, however with the observation reported by Kucera (6).

Whether the associations found between epilepsy and the variables studied are causal or not, is a type of question which is common to all case-control studies. It is not difficult to imagine that midwives and physicians may take a more detailed history from women who report some disease or who report antepartum complications. Also it may be imagined that women with epilepsy are more apt to report more completely on the complications during their pregnancy Neither is it unlikely that the observation of premature labour congenital malformation or low birth weight leads to a more thorough history taking in search of an "explanation" On the other hand, it is less likely that conduct of labour and complications during delivery should be more completely recorded for women with epilepsy than others. In fact the higher frequency of induction of labour and interventions during labour found in women with epilepsy coincides well with their higher frequency of complicated pregnancies. Moreover the higher frequency of antepartum complications, prematurity malformations and low birth weight, undoubtedly leads to higher perinatal and neonatal mortalities, as actually observed As pointed out in the section on Material and Methods information on deaths is derived from a source independent of the medical registration of births and this lends some support to the suggestion that the observed associations are cause and effect relationships.

This conclusion means, in practical terms, that women with epilepsy should be considered a high risk group in need of special attention during pregnancy and special care during labour Indeed, they should be included among those women to be selectively admitted to the clinics offering advanced obstetric services.

In scientific terms the cause and effect relationships suggested by the present study mean that further studies directed towards revealing the underlying or the more direct causal mechanism are warranted. The collection of detailed information on pregnancy in epileptics including medication in a reasonably large number of cases suggests itself as a necessary step

ACKNOWLEDGEMENTS

We thank the EDP-Section of the Medical Faculty University of Bergen for the data processing of the material, and in particular Mr Svein Pedersen for his assistance in programming. We also thank the Norwegian Agency for International Development for granting one of us (S L.B) a post-doctoral fellowship.

REFERENCES

1 Baptisti, A.: Epilepsy and pregnancy A review of the literature and a study of thirty-seven cases. Amer J Obstet Gynec 35 818, 1938
2. Bjerkedal, T & Bakketeig, L. Medical registration of births in Norway 1967–68. Some descriptive and analytical aspects. Institute of Hygiene and Social Medicine University of Bergen, Norway 1972.
3 Burnett, C. W F Survey of relation between epilepsy and pregnancy J Obstet Gynaec Brit Emp 53. 539 1946
4 Halter G Status epilepticus in pregnancy Wien Med Wschr 113 531 1963
5 Hedberg, E., Holmdahl, K. & Pehrson, S. On relationship between maternal health and congenital malformations. Acta Obstet Gynec Scand 46 378, 1967
6 Kucera, J P terms of congen tal anomalies in the off pring of omen exposed to different drugs and or chemicals during pregnancy Abstracts of papers presented at the first meeting of the European Teratology Society Cardiff Wales, April 13–16, 1971 Teratology 4 49., 1971
7 McClure J H Idiopathic epilepsy in pregnancy Summary of the literature nd clinical study of twenty patients. Amer J Obstet Gynec 70 296 1955
8 M lby F Medical registration of births, T Norske Laegefor 87 1085 1967
9 Sabin, M & Oxorn, H Epilepsy and pregnancy Obstet Gynec 7 175 1956
10. Suter J D Convulsions and coma in pregnancy Amer J Obstet Gynec 84 6 3 196.

Submitted fo publication Oct 10 197

Tor Bjerkedal
Institut of Hygiene and Social Medicine
University of Bergen
5000 Bergen
Norway

small-for-dates fetus by serial ultrasonic cephalometry. Lancet 2: 1002, 1971.

Donald, I. & Brown, T. G. Localisation using physical devices, radioisotopes and radiographic methods. I. Demonstration of tissue interfaces within the body by ultrasonic echo sounding. Brit J Radiol *34* 539. 1961.

Juurpälä, P. Ylöstalo, P. & Pystynen, P. Fetal head growth measured by ultrasound in the last few weeks of pregnancy in normal, toxaemic and diabetic women. Acta Obstet Gynec Scand *49*, 367. 1970.

Kratochwil, A. Ultraschalldiagnostik in Geburtshilfe und Gynäkologie. Georg Thieme Verlag, Stuttgart, 1968.

Lubchenco, Lula O. Hansman, Charlotte, Dressler, Marion & Boyd, Edith. Intrauterine growth as estimated from liveborn birth-weight data at 24 to 42 weeks of gestation. Pediatrics *32* 793. 1963.

8. Willocks, J. Donald, I., Campbell, S. & Dunsmore, I. R. Intrauterine growth assessed by ultrasonic foetal cephalometry. J Obstet Gynaec Brit Comm *74* 639, 1967.

9. Willocks, J. & Dunsmore, I. R. Assessment of gestational age and prediction of dysmaturity by ultrasonic fetal cephalometry. J Obstet Gynaec Brit Comm *78* 804, 1971.

Submitted for publication Oct. 18, 1972

Per Bergsjø
Bjarne Skauersvei 15
1347 Hosle
Norway

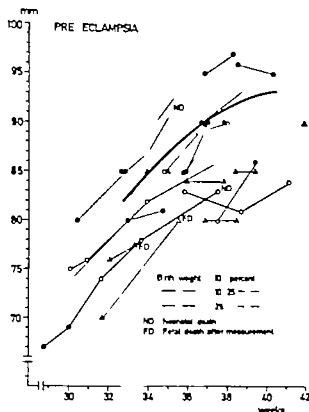


Fig 1 Biparietal diameters and gestational age i 35 patients with pre-eclampsia

black spots in the figure the biparietal diameters are scattered normally about the normal growth curve One neonate with a birth weight slightly above the average and a biparietal diameter well above the average died on the first day of life due to prematurity and hemorrhagic disease With the exception of one case with measurements above the normal average near term the serial measurements give no indication of growth retardation in any of the cases.

In groups *b* and *c* which include the 18 growth-retarded children marked with open spots in the figure the biparietal diameters in 16 cases were clearly below the average while one was slightly above and one touched the normal growth line. The measurements gave no clear distinction between groups *b* and *c* There were two fetal deaths and one neonatal death among the children below the 25th percentile The serial measurements indicated growth retardation in 4 cases, but these did not include the cases of perinatal death

DISCUSSION

We found that fetuses in the lower weight range at any given gestational age tended to have smaller biparietal diameters than those of normal weight. Serial measurements in the individual case were useful in confirming this tendency

The severity of pre-eclampsia is an important factor in the development of chronic placental insufficiency and thereby of fetal growth retardation We did not sub-classify our cases, but the selection of th material with most of the patients being in hospital for several weeks indicates that they lie in the upper range of severity Our conclusion agrees with that of Campbell & Dewhurst (3) but contradicts the results of Jouppila et al. (5) mentioned in the introduction. A discussion of reasons for this discrepancy is impossible without further information about the clinical series in question We have noticed that the average curve of normal growth presented by Jouppila et al (5) shows smaller biparietal diameters than other similar published curves (1 2 6 9), but if this is a persistent tendency due to the method it should not influence the results.

Although ultrasonic cephalometry gives very exact measurements one must allow for a certain degree of interpretation error on the part of the examiner One instance of apparent skull shrinkage in Fig. 1 may be an example of such error This operational error is not thought to affect the results in the present series, in which the classification of fetal growth retardation was done retrospectively

In the present series the assessment of growth indices would not have contributed to the prediction of fetal death but the serial measurements did indicate cessation of growth in some cases. The intervals between examinations in the individual patient were probably too long to be of great value in this respect. Serial measurements at weekly intervals, with predictions based on two successive growth indices, as proposed by Campbell & Dewhurst (3) appear to be the right approach.

REFERENCES

1 Brodtkorb C & Bergsjö P Bestemmelse ... kapudengalen ... ed hjelp ... hr lyd Nord Med 85 1569 1971

C mpbell, S Th prediction f fetal maturity by ultrasonic measurement f the biparietal di meter J Obstet Gynaec Brit C mm 76 603 1969

3 Campbell, S & Dewhurst, C J Diagnosis f the

small-for-dates fetus by serial ultrasonic cephalometry Lancet 2. 1002, 1971.

4 Donald, I. & Brown, T. G. Localisation using physical devices, radioisotopes and radiographic methods. I. Demonstration of tissue interfaces within the body by ultrasonic echo sounding. Brit J Radiol *34* 539 1961.

5. Jouppila, P. Ylöstalo, P. & Pystynen, P. Fetal head growth measured by ultrasound in the last few weeks of pregnancy in normal, toxaemic and diabetic women. Acta Obstet Gynec Scand *49* 367 1970.

6. Kratochwil, A.. Ultraschalldiagnostik in Geburtshilfe und Gynäkologie. Georg Thieme Verlag, Stuttgart, 1968

7 Lubchenko, Lula O. Hansman, Charlotte, Dressler, Marion & Boyd, Edith: Intrauterine growth as estimated from liveborn birth-weight data at 4 to 42 weeks of gestation. Pediatrics *34* 793 1963

8. Willocks, J. Donald, I. Campbell, S. & Dunsmore I. R.: Intrauterine growth assessed by ultrasonic foetal cephalometry. J Obstet Gynaec Brit Comm *74* 639 1967

9 Willocks, J. & Dunsmore, I. R.. Assessment of gestational age and prediction of dysmaturity by ultrasonic fetal cephalometry. J Obstet Gynaec Brit Comm *78* 804 1971.

Submitted for publication Oct. 18 1972

Per Bergsjø
Bjarne Skaus vei 15
1347 Hosle
Norway

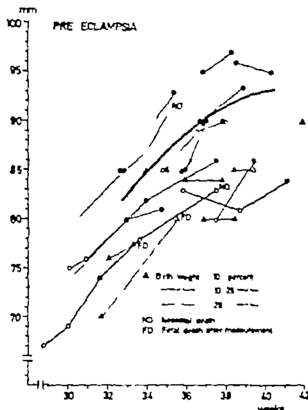


Fig 1 Biparietal diameters and gestational age in 35 patients with pre-eclampsia.

black spots in the figure the biparietal diameters are scattered normally about the normal growth curve One neonate with a birth weight slightly above the average and a biparietal diameter well above the average died on the first day of life *due to prematurity and hemorrhagic disease* With the exception of one case with measurements above the normal average near term the serial measurements give no indication of growth retardation in any of the cases.

In groups *b* and *c* which include the 18 growth retarded children marked with open spots in the figure the biparietal diameters in 16 cases were clearly below the average while one was slightly above and one touched the normal growth line The measurements gave no clear *distinction* between groups *b* and *c* There were two fetal deaths and one neonatal death among the children below the 25th percentile The serial measurements indicated growth retardation in 4 cases but these did not include the cases of perinatal death.

DISCUSSION

We found that fetuses in the lower weight range at any given gestational age tended to have smaller biparietal diameters than those of normal weight. *Serial measurements in the individual case* were useful in confirming this tendency

The severity of pre-eclampsia is an important factor in the development of chronic placental insufficiency and thereby of fetal growth retardation We did not sub-classify our cases, but the selection of the material with most of the patients being in hospital for several weeks indicates that they lie in the upper range of severity Our conclusion agrees with that of Campbell & Dewhurst (3) but contradicts the results of Jouppila et al. (5) *mentioned in the introduction.* A discussion of reasons for this discrepancy is impossible without further information about the clinical series in question. We have noticed that the average curve of normal growth presented by Jouppila et al. (5) shows smaller biparietal diameters than other similar published curves (1 2, 6 9), but if this is a persistent tendency due to the method it should not influence the results.

Although ultrasonic cephalometry gives very exact measurements one must allow for a certain degree of interpretation error on the part of the examiner One instance of apparent skull shrinkage in Fig 1 may be an example of such error This operational error is not thought to affect the results in the present series, in which the classification of fetal growth retardation was done retrospectively

In the present series the assessment of growth indices would not have contributed to the prediction of fetal death, but the serial measurements *did indicate cessation of growth in some cases.* The intervals between examinations in the individual patient were probably too long to be of great value in this respect. Serial measurements at weekly intervals, with predictions based on two successive growth indices as proposed by Campbell & Dewhurst (3) appear to be the right approach.

REFERENCES

1 Brodtkorb C & B. jo P Bestemmelse ... range ... sk pelengde ... ed hjelp ... ltralyd Nord Med 85 1 69 1971
Campbell, S Th predict ... f fetal maturity by ltrasonic me sureme t of the biparietal di meter J Obstet Gynaec Brit C mm 76 603 1969
3 Campbell, S & Dewhurst, C J Diagnosis f the

small-for-dates fetus by serial ultrasonic cephalometry Lancet 2: 1002, 1971.

4. Donald, I. & Brown, T G. Localisation using physical devices, radioisotopes and radiographic methods I. Demonstration of tissue interfaces within the body by ultrasonic echo sounding. Brit J Radiol *34* 539 1961

5. Jouppila, P Ylöstalo, P & Pystynen, P Fetal head growth measured by ultrasound in the last few weeks of pregnancy in normal, toxaemic and diabetic women. Acta Obstet Gynec Scand *49* 367 1970.

6. Kratochwil, A.: Ultraschalldiagnostik in Geburtshilfe und Gynäkologie. Georg Thieme Verlag, Stuttgart, 1968

7. Lubchenco, Lula O., Hansman, Charlotte, Dressler Marion & Boyd, Edith. Intrauterine growth as estimated from liveborn birth-weight data at 24 to 42 weeks of gestation Pediatrics *32* 793 1963

8. Willocks, J Donald, I Campbell, S. & Dunsmore, I. R. Intrauterine growth assessed by ultrasonic foetal cephalometry J Obstet Gynaec Brit Comm *74* 639 1967

9. Willocks, J & Dunsmore, I. R. Assessment of gestational age and prediction of dysmaturity by ultrasonic fetal cephalometry. J Obstet Gynaec Brit Comm *78* 804, 1971.

Submitted for publication Oct 18 1972

Per Bergsjø
Bjarne Skauesvei 15
1347 Hosle
Norway

Acta Obstet Gynec Scand 52. 253–258 1973

POSTOVULATORY ENDOMETRIAL DEVELOPMENT IN WOMEN WITH I.U.D.

Hans Henrik Nicolaisen, Henning Pedersen, Eigil Guttorm and Heinrich Rebbe

From the University Department of Gynaecology and Obstetrics, Øresundshospitalet (Heads: G. Stakemann and F. Lundvall), the University Institute of Pathological Anatomy (Heads: G. Teilum and E. Jensen), and the University Department of Gynaecology and Obstetrics, Rigshospitalet (Heads: D. Trolle and B. Sørensen), Copenhagen, Denmark

Abstract. Histological "dating" of endometrial biopsy specimens within the postovulatory period was performed in a group of normal women having an I.U.D. and another group of normal women without an I.U.D. In both groups the time of ovulation was determined by basal temperature measurement. By comparing the "histological" data with the actual point of time in the cycle, given in days after the ovulation, the group of women with an I.U.D. showed an average retardation of 2 days. In the control group the average retardation was zero days. The difference between the two groups was statistically significant. The group with an I.U.D. presented focal stromal changes, which should be investigated further with a view to possible endocrine secretion. The results of the present investigation of a fairly small series show that the contraceptive effect of an I.U.D. may be due to delayed endometrial development.

Within veterinary medicine the contraceptive effect of an intra-uterine foreign body has been known for more than 2000 years. In humans this sort of contraception has been employed for more than 40 years without, however, the mechanism of action having been definitely established.

In various reviews the results of experimental studies on different animal species are reported (5, 6, 8). It appears that the mechanism of contraceptive action differs from one species to another. Establishment of a simple mechanism is impossible on the basis of experimental animal studies, and it is hardly permissible to draw any conclusions with regard to the conditions in the human female.

Within recent years various reports have been published, which all bear out the hypothesis that an I.U.D. (intra-uterine device) causes a delayed development and maturation of the human endometrium, thus rendering normal nidation impossible.

In a—certainly small—series kept under observation pregnanediol was found to be excreted in diminished amounts after insertion of an I.U.D. (7).

Comprehensive studies of the vaginal cytology suggest the same mechanism (11).

Finally, the results of biochemical analyses of endometrial tissue of women with an I.U.D. support the diagnosis of retarded maturation (3).

Histological assessment of the morphology of the endometrium is likely to throw further light on a possibly retarded maturation. Rock & Bartlett (10) showed several years ago that each postovulatory day of the cycle presents its separate well-defined, histological picture, and that accordingly an endometrial biopsy specimen can be "dated" with fairly great accuracy. Several investigators have, in fact, studied the endometrial development in women with an I.U.D. in this manner, but their results do not all agree (1, 12, 14). In the above investigations the development of the endometrium is given in relation to the first day of menstruation. This may account for the differing results, because the time of ovulation may vary rather considerably in relation to the first day of menstruation.

Present Investigations

In the present study we have aimed at elucidating the maturation of the endometrium by histological "dating" starting from the time of ovula-



tion. This was fixed by measuring the basal temperature. That ovulation had taken place was verified by determining the pregnanediol concentration in urine immediately before endometrial biopsy was performed. Comparison of the histological point of time, given in days after the ovulation with the actual date of the biopsy affords a chance of disclosing a possible retardation of maturation.

The total series investigated comprised normal women with an I U D and normal women without an I U D all examined under identical circumstances. By comparing the results achieved within the two groups we should be able to determine whether retardation occurs in women with an I U D

MATERIAL AND METHOD

From women with an I U D ("Lippes loop) a group was selected on the basis of the following criteria (a) The I U D had been inserted at least 1 y ar previously (b) menstruation was regular with cycle of from 27 to 3 days, (c) the patients presented no gynaecological signs or symptoms, and (d) they had all previously been delivered after uncomplicated pregnancy

As controls, 14 normal women were chosen, all examined on identical lines owing to involuntary childlessness, and in all cases infertility of the male partner explained their failure to conceive.

The basal temperature curve showed the ovulation time



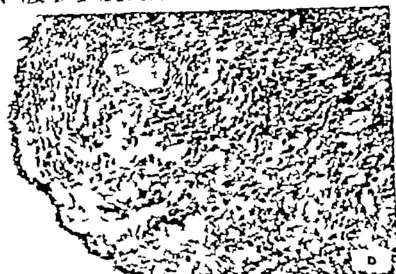


Fig. 3. Endometrial biopsy specimens from women with an IUD. (A) Normal endometrium 5 days after ovulation (H & E, × 135). (B) Focal pseudodecidua-like change in the same biopsy specimen (H & E, × 135). (C) Abundant PAS-positive material in glandular epithelium (section corresponding to A), note stromal cells without PAS-positive substance (PAS, × 135). (D) Focal pseudodecidua-like reaction (section corresponding to B), abundant PAS-positive substance in stromal cells (PAS, × 135).

the first day with definite rise to a new level having been fixed as the first postovulatory day.

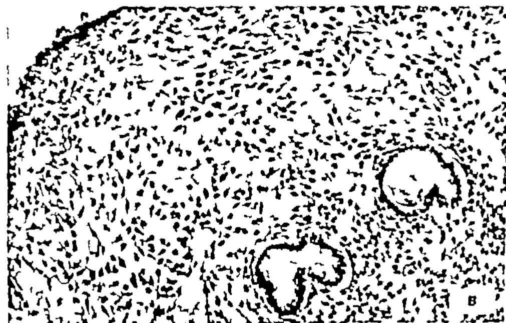
Further, the pregnanediol excretion was determined in urine, collected for 24 hours within the period of 6–8 days after the expected ovulation. The analyses were performed using O. Bang & N. C. Hansen's modification of Klopper's method (4). An excretion of 2 mg/24 hours or more was considered as proof that normal ovulation had taken place (standard deviation 0.15 mg/24 hours, and day to day variation 0.4 mg/24 hours).

The histological dating was based on the bases indicated by Rock & Bartlett (10) and further developed by Noyes et al. (9).

The biopsy specimens were fixed in 96% ethanol. The usual paraffin technique was employed, and the following stains were used: haematoxylin-eosin, Van Gieson, alcian blue, and PAS-stain.

The dating is based on estimation of the gland morphology, the height of the glandular epithelium, the location of the nuclei in relation to the basal membrane, possible occurrence of secretion vacuoles in the cytoplasm, occurrence of mitoses, sizes of the glandular





tion. This was fixed by measuring the basal temperature That ovulation had taken place was verified by determining the pregnanediol concentration in urine immediately before endometrial biopsy was performed. Comparison of the histological point of time, given in days after the ovulation with the actual date of the biopsy affords a chance of disclosing a possible retardation of maturation.

The total series investigated comprised normal women with an I U D and normal women without an I U D all examined under identical circumstances. By comparing the results achieved within the two groups we should be able to determine whether retardation occurs in women with an I U D

MATERIAL AND METHOD

From women with an I U D ("Lippes loop) a group was selected on the basis of the following criteria (a) The I U D had been inserted at least 1 year previously (b) menstruation was regular with a cycle of from [illegible] to [illegible] days, (c) the patients presented no gynaecological symptoms, and (d) they had all previously been delivered after uncomplicated pregnancy

As controls, 14 normal women were chosen, all examined on identical lines owing to involuntary childlessness, and in [illegible] cases infertility of the male partner explained their failure to conceive.

The basal temperature curves showed the ovulation time

Table II. *Fourteen women without I.U.D.*

Patient no	Pregnanediol excretion (mg/24 hours)	Cycle day	Days after ovulation	Histological data (days after ovulation)	Retardation (number of days)
1	4.6	17	5	6	−1
2	2.8	19	6	5.5	+0.5
3	2.4	24	9	7.5	+1.5
4	2.5	24	8	10	−2
5	2.4	26	11	12	−1
6	4.1	22	10	10	0
7	3.2	22	8	10	−2
8	2.1	23	8	8.5	−0.5
9	1.8	23	6	5.5	+0.5
10	4.2	19	8	7	+1
11	4.3	24	6	6.5	−0.5
12	2.4	24	8	8	0
13	3.1	23	11	9	+2
14	2.8	23	8	7	+1

Average retardation (mean), 0

specimens from women with an I.U.D. displayed superficial, focal changes of the stroma. Nests of enlarged, pseudodecidual-like cells could be demonstrated, while the remaining part showed development corresponding to a date preceding that at which the pseudodecidual reaction normally can be observed. Fig. 1 illustrates a biopsy specimen dated to five days after the time of ovulation. Within a single area enlarged stromal cells are seen, which have abundant, PAS-positive cytoplasm. Normally pseudodecidual reaction is first noticeable about the ninth day after the ovulation.

DISCUSSION

The investigation showed an average retardation of 2 days in the group with an I.U.D. against zero days in the control group. It has been stated previously (15) that retardation of more than 2 days interferes with nidation, with a consequent reduction in fertility. Hence, there is reason to suppose that this retardation is one cause of the contraceptive effect of the I.U.D.

The mechanism of the endometrial retardation may possibly be diminished corpus luteum activity as suggested by Tauscher et al. (1969). It should be mentioned, however, that in our small series with only one pregnanediol estimation to verify that ovulation had taken place, no differences in pregnanediol excretion could be found between the two groups.

It has been suggested that the poorly developed endometrium might interfere with corpus luteum secretion via hormonal or neurogenic factors, a hypothesis which has to some extent been borne out by the results of animal experiments (2). Mention should also be made of the focal changes in the endometrial stroma noticed in the present material. At a certain point of time within the cycle, prior to the development of a normal pseudodecidual reaction, foci were noticed having large, decidua-like cells with PAS-positive cytoplasm. Similar changes have never been observed in endometria of women without an I.U.D. in our series. The explanation may be a direct mechanical action on the endometrium. Whether these cells are responsible for the secretion of some endometrial retarding factor is another question, which cannot yet be answered, but the possibility should be mentioned.

Summarizing we may say that the relatively small series available allows no extensive conclusions. Much larger series must be studied before we can say for certain whether the retardation is real. It will also be a matter of interest to investigate whether among women with an I.U.D. we can separate out a group showing no retardation in which pregnancy occurs more frequently. Finally the focal changes of the stroma ought to be subjected to further study by electron microscopy and histochemical methods with view to clarifying the question of endocrine secretion.

Table I *Fourteen women intw I U D*

Patient no.	Pregnanediol excretion (mg/24 hours)	Cycle day	Days after ovulation	Histological date (days after ovulation)	Retardation (number of days)
1	.9		10	7.5	+.5
2	2.6	2.	9	8.5	+0.5
3	1.9	22	7	.5	+4.5
4	2.9	22	11	9	+2
5	2.5	23	7	5	+2
6	3 1	19	7	6.5	+0.5
7	3 7	24	11	8.5	+2.5
8	2.9	23	4	3	+1
9	1 9	23	8	6	+2
10	2.1	21	5	3	+
11	5 4	20	3	2	+1
12	2.5	26	10	6	+4
13	4 0	21	7	6.5	+0.5
14	2.4	24	11	7.5	+3.5

Average retardation (mea), +2.0

lumina, and the content of acid mucopolysaccharides, height of the surface epithelium, sizes of the stromal cells, stromal oedema, possible presence of pseudodecid u l reaction, and the arteriolar structure

During the first 5 days after the ovulation the sites of the secretion vacuoles and the location of the nuclei in the glandular epithelium are especially characteristic. From the sixth to the ninth day the gland morphology and in particular the content of acid mucopolysaccharides in the lumen and presence of stromal oedema are importa t aids towards the dating. Finally the development of the pseudodecidual reaction and the structure of the arterioles are of importance in the remaining part of the cycle

As stated above histological pictures ha e been set up one corresponding to each day after the ovulation. Note however that not only do individual differences occur from one biopsy specimen to another within the normal range but the development of glands and stroma also varies within the same biopsy pecimen. On this account the dating itself will alway be based on a rough estimate.

In the present investigation we aimed at referring the histological picture as far as possible to a single day This was, however impossible in several cases, where th. smallest possible t me interval was given instead, e.g. *endometrium 7–8 days after the time of ovulation.*

The histological specimens were all examined by one of us (H. P) The indications of time are based exclusiv ly on the examined biopsy specimens, nd it should be emphasized that in all cases the ovulation time wa unknown to the pathologist.

RESULTS

A total of 25 women with an I U D completed the examination programme Of these 11 had to be ruled out for the following reasons. five on a count of a too low pregnanediol excretion (ranging from 0.4 to 1 6 mg/24 hours) one owing to a useless temperature curve two because the biopsy specimen was taken too early (in both cases at proliferation phase) and three because the biopsy specimens were unfit.

Useful data were thus available from 14 women with an I U D whose values have been set out in Table I Retardation was found in all these cases ranging from 0.5 to 4 5 days In 5 cases it exceeded 2 days. (Where the retardation is given in decimals the pathologist was unable to indicate a single day in the cycle The values ac cordingly represent the average of the stated time intervals.) The times at which biopsy specimens were taken varied more than intended as some of the women found it inconvenient to attend when requested.

The results of the examination of the control group are shown in Table II In some cases nega tive values have been given for the retardation indicating that the histological picture was judged to be at a more developed stage than correspond ing to the actual point of time In the control group the retardation wa evenly distributed round zero days, ranging from to + days.

Using Student s test the two groups were found to differ significantly with regard to mean re tardation (t 4 3693 corresponding to p 0 001)

The tables give the values of the pregnanediol ex retion in both groups, and the values do not seem to differ

Further half of the suitable endometrial biopsy

Table II. *Fourteen women without I.U.D*

Patient no.	Pregnanediol excretion (mg/24 hours)	Cycle day	Days after ovulation	Histological date (days after ovulation)	Retardation (number of days)
1	4.6	17	5	6	−1
2	2.8	19	6	5.5	+0.5
3	2.4	24	9	7.5	+1.5
4	2.9	24	8	10	−2
5	2.4	26	11	12	−1
6	4.1	23	10	10	0
7	3.2	22	8	10	−2
8	2.3	23	8	8.5	−0.5
9	1.8	23	6	5.5	+0.5
10	4.2	19	8	7	+1
11	4.3	24	6	6.5	−0.5
12	2.4	24	8	8	0
13	3.1	23	11	9	+2
14	2.8	23	8	7	+1

Average retardation (mean), 0

specimens from women with an I.U.D. displayed superficial, focal changes of the stroma. Nests of enlarged, pseudodecidual-like cells could be demonstrated, while the remaining part showed development corresponding to a date preceding that at which the pseudodecidual reaction normally can be observed. Fig. 1 illustrates a biopsy specimen dated to five days after the time of ovulation. Within a single area enlarged stromal cells are seen, which have abundant, PAS-positive cytoplasm. Normally pseudodecidual reaction is first noticeable about the ninth day after the ovulation.

DISCUSSION

The investigation showed an average retardation of 2 days in the group with an I.U.D. against zero days in the control group. It has been stated previously (13) that a retardation of more than 2 days interferes with nidation, with a consequent reduction in fertility. Hence, there is reason to suppose that this retardation is one cause of the contraceptive effect of the I.U.D.

The mechanism of the endometrial retardation may possibly be diminished corpus luteum activity as suggested by Fancher et al. (1969). It should be mentioned, however, that in our small series, with only one pregnanediol estimation to verify that ovulation had taken place, no differences in pregnanediol excretion could be found between the two groups.

It has been suggested that the poorly developed endometrium might interfere with corpus luteum secretion via hormonal or neurogenic factors, a hypothesis which has to some extent been borne out by the results of animal experiments (2). Mention should also be made of the focal changes in the endometrial stroma noticed in the present material. At a certain point of time within the cycle prior to the development of a normal pseudodecidual reaction, foci were noticed having large, decidua-like cells with PAS-positive cytoplasm. Similar changes have never been observed in endometria of women without an I.U.D. in our series. The explanation may be a direct mechanical action on the endometrium. Whether these cells are responsible for the secretion of some endometrial retarding factor is another question, which cannot yet be answered, but the possibility should be mentioned.

Summarizing we may say that the relatively small series available allows no extensive conclusions. Much larger series must be studied before we can say for certain whether the retardation is real. It will also be a matter of interest to investigate whether among women with an I.U.D. we can separate out a group showing no retardation in which pregnancy occurs more frequently. Finally the focal changes of the stroma ought to be subjected to further study by electron microscopy and histochemical methods with a view to clarifying the question of endocrine secretion.

Table I *Fourteen women with I U D*

Patient no	Pregnanediol excretion (mg/24 hours)	Cycle day	Days after ovulation	Histological date (days after ovulation)	Retardation (number of days)
1	2.9	[illegible]	10	7.5	+[illegible]5
	2.6	2[illegible]	9	8.5	+0.5
3	1 9	22	7	2.5	+4.5
4	2.9	22	11	9	+
5	2.5	23	7	5	+2
6	3 1	19	7	6.5	+0.5
7	3 7	4	11	8.5	+2.5
8	2.9	23	4	3	+1
9	1.9	23	8	6	+2
10	2.1	21	5	3	+2
11	5 4	20	3	2	+1
12	2.5	26	10	6	+4
13	4 0	21	7	6.5	+0.5
14	2.4	24	11	7.5	+3.5

Average retardation (mean), +2.0

lumina, and the content of acid mucopolysaccharides, height of the surface epithelium, sizes of the stromal cells, stromal oedema, possible presence of pseudodecid ual reaction, and the arteriolar structure

During the first 5 days after the ovulation the sites of th secretion vacuoles and the location of the nuclei in the glandular epithelium are especially characteristic. From the sixth to the ninth day the gland morphology and in particular the content of acid mucopolysaccharides in the lumen and presence of stromal oedema are important aids towards the dating. Finally the development of the pseudodecidual reaction and the structure of the arterioles are of importance in the remaining part of the cycle.

As stated above hist logical pictures ha e been set up, one corresponding to each day after the ovulation. N te however that not only do indi idual differences occur from one biopsy specimen to another within the normal range but the development of glands and stroma also varies within the same biopsy specimen. On this account the dating itself will always be based on a rough estimate

In the present investigation we aimed at referring the histological picture as f as possible to a single day This was, however impossible in several cases, where th smallest possible time interval was given instead, e.g. endometrium 7–8 days after the time of ovulation.

The histological specimens were all xamined by one of us (H. P) The indications f time re based xclusively on the examined biopsy specimens, and t should be emphasized that in all cases the ovulation time was unknown to the pathologist.

RESULTS

A total of 25 women with an I U D completed the examination programme Of these 11 had to be ruled out for the following reasons. five on ac count of a too low pregnanediol excretion (ranging from 0 4 to 1 6 mg/24 hours) one owing to a useless temperature curve two because the biopsy specimen was taken too early (in both cases at proliferation phase) and three because the biopsy specimens were unfit.

Useful data were thus available from 14 women with an I.U D whose values have been set out in Table I Retardation was found in all these cases ranging from 0 5 to 4.5 days in 5 cases it exceeded 2 days. (Where the retardation is given in decimals the pathologist was unable to indicate a single day in the cycle The values ac cordingly represent the average of the stated time intervals.) The times at which biopsy specimens were taken varied more than intended as some of the women found it inconvenient to attend when requested

The results of the examination of the control group are shown in Table II In some cases nega tive values have been given for the retardation indicating that the histological picture was judged to be at a more developed stage than correspond ing to the actual point of time In the control group the retardation was evenly distributed round zero days ranging from − to + days

Using Student s test the two groups were found to differ significantly with regard to mean re tardation (t 4 3693 corresponding to p 0 001)

The tables give the values of the pregnanediol ex retion in both groups and the values do not seem to differ

Further half of the uitable endometrial biopsy

Acta Obstet Gynec Scand 52, 259–268 1973

PREMATURE OVARIAN FAILURE

J Starup and V Sele

From the Department of Obstetrics and Gynaecology Y (Heads: Dyre Trolle, Mogens Osler Børge Sørensen and Vagn Sele), Rigshospitalet Copenhagen, Denmark

Abstract. Twenty-six patients with premature ovarian failure (last menstrual period before the age of 30 years) were evaluated clinically and endocrinologically and, in addition, ovarian biopsies were obtained in 15 out of the 26 patients. Only 1 of the patients revealed a family history of early menopause. The typical menstrual pattern indicated a normal age at menarche with regular periods for various length of time followed either by amenorrhoea abruptly or by a period of oligomenorrhoea before the onset of amenorrhoea. All patients showed normal or slightly underdeveloped secondary sexual characteristics, and it should be noted that 2 patients had been pregnant previously.

It was characteristic of all patients that the excretion of total gonadotrophins was constantly increased and that the excretion of total oestrogens was low whereas both the adrenal and thyroid function were normal. All patients were X-chromatin positive.

In 13 out of the 15 patients, in whom ovarian biopsies were obtained, the histological examination revealed a normal stroma without or with very few primordial and atretic follicles, a picture very similar to that characteristic of the postmenopausal ovary. In the last 2 patients, however, the histological examination showed a quite different picture, viz. a normal stroma with numerous primordial and primary follicles and even a few growing follicles. In spite of these morphologically normal follicles, it was not possible to stimulate the follicular development and to induce ovulation even with very high doses of human gonadotrophins. It is, therefore, concluded that the presence of a few growing follicles in the ovaries does not seem to exclude the diagnosis of premature ovarian failure.

Only 14 out of the 26 patients had the typical symptoms of the climacteric, and even in these cases the intensity is only of a mild to moderate degree. It is generally agreed that all patients with symptoms should receive substitution therapy but the increased risk of ischaemic heart disease and osteoporosis, together with the unquestionable psychological effect of having regular menstrual bleeding, have led us to the conclusion that this therapy is indicated in all cases of premature ovarian failure. Cyclical treatment either with sequential preparations or with oestrogens alone is the treatment of choice.

Physiological exhaustion of the ovarian follicles in women occurs as a rule, at an age between 40 and 55 years, and the result is inevitably a loss of both the reproductive capacity and of the greater part of the ovarian hormonal activity. According to Millot & Daux (13) only 3.7% of all women pass the menopause before the age of 40 years, whereas Thierraud-Perrier (22) and Stepancovsky (20) found a percentage of 8.4 and 11.0 respectively.

In very rare cases ovarian function ceases unusually early and this condition has been named premature menopause or better premature ovarian failure. We think the term "premature ovarian failure" should be preferred, because it describes the syndrome clinically and pathologically and still does not necessarily imply aging. Unfortunately there is no complete agreement on the definition of this syndrome, especially with regard to the upper age limit. The majority of investigators (1, 3, 11, 12, 14, 16, 24) have used an age limit of 35 years and a few authors (6, 15) even a limit of 40 years, whereas we, in agreement with some other investigators (2, 5, 8, 17, 18) have preferred the age limit of 30 years to be certain that all cases of early menopause within normal physiological limits are excluded.

The purposes of the present study are (*a*) to describe in detail the clinical and hormonal findings in patients with premature ovarian failure, (*b*) to describe and discuss ovarian morphology in these patients, and finally (*c*) to discuss whether all patients should receive substitution therapy and if so what kind of treatment one should recommend.

REFERENCES

1 Aguilera, E. Effect of the IUD on the endometrium. *I* Proceedings of the Eighth International Conference of the International Planned Parenthood Federation, Santiago, Chile 1967 p. 3..3 International Planned Parenthood Federation, Hertford, England, 1967

2. Bland, K. P & Donovan, B. T Neural and humoral stimuli from the uterus and control of ovarian func tion. *In* Egg Implantation p. 29 Ciba Foundation, London, 1966.

3 Bonney W A., Glasser S. R., Clewe, T H., Noyes, R. W & Cooper C. L. Endometrial response to the intrauterine device. Amer J Obstet Gynec 96. 101 1966.

4 Buus, O. & Hansen, N C. Bestemmelse af pregnandiol i døgnurin fra normale Ugeskr Laeg *1 8* 10 1966.

5 Corfman, P A. & Segal, S. J. Biologic effects of intrauterine devices. Amer J Obstet Gynec *100* 448, 1968

6. Eckstein, P Intrauterine contraceptive devices. The scientific background. Proc Roy Soc Med *60* 39., 1967

7 Fancher G L., Ellegood, J O Mahesh, C. B. & Greenblatt, R. B. Urinary estrogens and pregnanediol before and after insertion of an intrauterine contracepti e device. Amer J Obstet Gynec *104* 502, 1969

8 Kar A. B. Mechanism of action of intrauterine contraceptive devices *In* Proceedings of the Eighth International Conference of the International Planned Parenthood Federation, Santiago, Chile 1967 p. 3?3 International Planned Parenthood Federation, Hertford, England, 1967

9 Noyes, R. W Hertig, A. T & Rock, J Dating of endometrial biopsy Fertil Steril *1* 3, 1950.

10. Rock J & Bartlett, M K. Biopsy studies of human endometrium. J Amer Med Ass *108* ?02., 1937

11 Sammour M. B., Iskander S. O. & Rifai, S. F Combined histologic and cytologic study of intrauterine device. Amer J Obstet Gynec *93* 946, 1967

12. Sujan-Tejuja, S., Virick, R. K. & Malkani, P K. Uterine histopathology in the presence of intrauterine devices. *In* Intra-Uterine Contraception, p 172. Excerpta Medica Foundation, Belgium, 1965

13 Williams, W W., *Sterility* third edition, p. ?? Published by the author Springfield, USA, 1964.

14 Wilson, J R., Ledger W J & Andros, G. J The effect of an intrauterine contraceptive device on the histologic pattern of the endometrium. Amer J Obstet Gynec *93* 802, 1965

Submitted for publication October 18 1972

Hans Henrik Nicolaisen
University Dept. of Gynaecology and Obstetrics
Øresundshospitalet
Copenhagen
Denmark

Acta Obstet Gynec Scand 52: 259–268 1973

PREMATURE OVARIAN FAILURE

J Starup and V Sele

From the Department of Obstetrics and Gynaecology Y (Heads: Dyre Trolle, Mogens Osler Børge Sørensen and Vagn Sele), Rigshospitalet, Copenhagen, Denmark

Abstract Twenty-six patients with premature ovarian failure (last menstrual period before the age of 30 years) ere evaluated clinically and endocrinologically and, in addition, ovarian biopsies were obtained in 15 out of the 26 patients. Only 1 of the patients revealed family history of early menopause. The typical menstrual pattern selected normal age at menarche ith regular periods for various length of time followed either by amenorrhoea abruptly or by a period of oligomenorrhoea before the onset of amenorrhoea. All patients showed normal or slightly underdeveloped secondary sexual characteristics, and should be noted that 2 patients had been pregnant previously

It was characteristic of all patients that the excretion of total gonadotrophins was constantly increased and that the excretion of total oestrogens was low whereas both the adrenal and thyroid function were normal. All patients were X-chromatin positive.

In 13 out of the 15 patients, in hom ovarian biopsies were obtained, the histological examination revealed normal stroma ithout or with very few primordial and atretic follicles, picture very similar to that characteristic of the postmenopausal ovary In the last 2 patients, however, the histological examination showed quite different picture, viz. normal stroma with numerous primordial and primary follicles and even few growing follicles In spite of these morphologically normal follicles, was not possible to stimulate the follicular development and to induce ovulation even with very high doses of human gonadotrophins It is, therefore concluded that the presence of few growing follicles in the ovaries does not seem to exclude the diagnosis of premature ovarian failure

Only 14 out of the 26 patients had the typical symptoms of the climacteric, and even in these cases the severity as only of mild to moderate degree It is generally agreed that all patients with symptoms should receive substitution therapy but the increased risk of ischaemic heart disease and osteoporosis, together with the unquestionable psychological effect of having regular menstrual bleeding, have led us to the conclusion that this therapy is indicated in all cases of premature ovarian failure Cyclical treatment either with sequential preparation or ith oestrogens alone is the treatment of choice.

Physiological exhaustion of the ovarian follicles in women occurs, as a rule, at an age between 40 and 55 years, and the result is inevitably a loss of both the reproductive capacity and of the greater part of the ovarian hormonal activity According to Millot & Daux (13) only 3.7% of all women pass the menopause before the age of 40 years, whereas Thieraud-Perrier (22) and Ste pancovsky (20) found a percentage of 8.4 and 11.0 respectively

In very rare cases ovarian function ceases unusually early and this condition has been named premature menopause or better premature ovarian failure We think the term "premature ovarian failure" should be preferred, because it describes the syndrome clinically and pathologically and still does not necessarily imply aging. Unfor tunately there is no complete agreement on the definition of this syndrome especially with re gard to the upper age limit. The majority of investigators (1 3 11 1., 14 16, 24) have used an age limit of 35 years and a few authors (6, 15) even a limit of 40 years, whereas we, in agreement with some other investigators (2, 5 8 17 18) have preferred the age limit of 30 years to be certain that all cases of early menopause within normal physiological limits are excluded

The purposes of the present study are (*a*) to describe in detail the clinical and hormonal findings in patients with premature ovarian failure, (*b*) to describe and discuss ovarian morphology in these patients, and finally (c) to discuss whether all patients should receive substitution therapy and if so, what kind of treatment one should recommend

REFERENCES

1 Aguilera, E., Effect of the IUD on the endometrium. *In* Proceedings of the Eighth International Conference of the International Planned Parenthood Federation, Santiago, Chile 1967 p. 3_3 International Planned Parenthood Federation, Hertford, England, 1967
_ Bland, K. P & Donovan, B. T Neural and humoral stimuli from the uterus and control of ovarian function. *In* Egg Implantation p. 29 Ciba Foundation, London, 1966.
3 Bonney W A., Glasser S. R., Clewe T H Noyes, R. W & Cooper C. L., Endometrial response to the intrauterine device Amer J Obstet Gynec *96* 101 1966.
4 Buus, O. & Hansen, N C. Bestemmelse af pregnandiol i døgnurin fra normale. Ugeskr Laeg *1?8* 10, 1966.
5 Corfman, P A. & Segal, S. J Biologic effects of intrauterine devices. Amer J Obstet Gynec *100* 448 1968.
6. Eckstein, P *Intrauterine contraceptive devices.* The scientific background. Proc Roy Soc Med *60* 392, 1967
7 Faucher G. L., Ellegood, J O., Mahesh, C. B. & Greenblatt, R. B., Urinary estrogens and pregnanediol before and after insertion of an intrauterine contraceptive device. Amer J Obstet Gynec *104* 502, *1969*
8 Kar A. B., Mechanism of action of intrauterine contraceptive devices. *In Proceedings of the Eighth International Conference of the International Planned Parenthood Federation, Santiago, Chile 1967 p ?41 International Planned Parenthood Federation, Hertford, England, 1967
9 Noyes, R. W Hertig, A. T & Rock, J Dating of endometrial biopsy Fertil Steril *1* 3 1950.
10. Rock, J & Bartlett, M. K., Biopsy studies of human endometrium. J Amer Med Ass *108* ?022, 1937.
11 Sammour M B., Iskander S. G. & Rifai, S. F Combined histologic and cytologic study of intrauterine device Amer J Obstet Gynec *95* 946 196?
1_ Sujan-Tejuja, S., Virick, R. K. & Malkani, P K. Uterine histopathology in the presence of intrauterine devices. *In Intra Uterine Contraception*, p. 172. Excerpta Medica Foundation, Belgium, 1965
13 Williams, W W *Sterility* third edition, p. ?? Published by the author Springfield, USA, 1964.
14 Wilson, J R., Ledger W J & Andros, G J The effect of an intrauterine contraceptive device on the histologic pattern of the endometrium. Amer J Obstet Gynec *93* 802, 1965

Submitted for publication Octobe 18 1972

Hans Henrik Nicolaisen
University Dept. of Gynaecology and Obstetrics
Øresundshospitalet
Copenhagen
Denmark

15	27	[illegible]	[illegible]	[illegible]	Regular until 24 years old, then oligomenorrhoea	26	10	Normal	Mild hot flushes and sweatings	N	0	Yes (2 y.)
16	28	162	62.4	14	Regular until 26 years old	26	22	Normal	No	Cousin has primary amenorrhoea	Normal delivery 18 years old	No
17	25	167	92.5	13	Regular until 23 years old, then oligomenorrhoea	24	13	Normal	No	No	0	No
18	24	164	71.5	12	Regular until 19 years old	19	40	Normal	No	No	0	No
19	29	168	56.8	11	Regular until 16 years old	16	44	Normal	Mild hot flushes and palpitations	No	0	Yes (1 y.)
20	24	151	50.7	14	Always severe oligomenorrhoea	23	12	Normal	No	No	0	No
21	28	178	75.0	12	Always slight oligomenorrhoea	27	8	Normal	No	No	0	Yes (3 y.)
22	26	169	64.5	14	Regular until 16 years old	16	115	Slightly under-developed	Moderate hot flushes and sweatings	No	0	Yes (5 y.)
23	30	160	50.0	13	Regular until 24 years old, then oligomenorrhoea	29	14	Normal	Mild hot flushes and sweatings	No	0	No
24	26	163	90.0	15	Regular until 18 years old, then oligomenorrhoea	23	34	Normal	Mild hot flushes and sweatings	No	0	N
25	24	155	69.5	14	Regular until 19 years old, then oligomenorrhoea	23	12	Normal	Moderate hot flushes and headaches	No	0	Yes (2 .)
26	26	154	45.1	13	Regular until 24 years old	24	27	Normal	Irritability and headaches	N	Normal delivery 19 years old	No

Table 1 *Clinical data for 26 patients with premature ovarian failure*

Patient no.	Age at admission (y)	Height (cm)	Weight (kg)	Menarche (y)	Previous menstrual pattern	Age at last menstruation (y)	Duration of secondary amenorrhoea (mo.)	Secondary sexual characteristics	Climacteric complaints	Disposition in family	Previous pregnancies	Problem of sterility
1	18	150	39.3	11	Regular until 12½ years old, then oligomenorrhoea	13	60	Slightly under developed	Moderate hot flushes and sweatings	No	0	No
2	30	155	57.0	14	Regular until 29 years old	29	10	Normal	No	No	0	Yes (3 y)
3	30	157	71.0	13	Regular until 17½ years old, then oligomenorrhoea	18	140	Normal	Moderate hot flushes and sweatings	No	0	Yes (2 y)
4	31	160	59.5	14	Always slight oligomenorrhoea	27	48	Normal	No	No	0	Yes (3 y)
5	23	160	47.7	14	Regular until 18 years old, then oligomenorrhoea	22	14	Normal	Moderate hot flushes and sweatings	No	0	No
6	27	175	71.0	17	Regular until 25 years old	25	22	Normal	No	No	0	Yes (3 y)
7	[illegible]0	154	53.0	14	Regular until 15 years old	15	60	Slightly under developed	No	No	0	No
8	28	164	62.0	15	Always slight oligomenorrhoea	26	21	Normal	No	No	0	No
9	[illegible]6	156	48.4	11	Regular until 21 years old, then oligomenorrhoea	22	44	Normal	Mild hot flushes and tiredness	No	0	Yes (3 y)
10	26	171	72.5	15	Regular until 22 years old	22	48	Normal	No	Mother and grandmother in early menopause	0	Yes (2 y)
11	21	165	54.1	14	Regular until 19 years old	19	16	Normal	No	No	0	No
12	22	169	63.0	13	Regular until 18 years old, then oligomenorrhoea	21	18	Normal	Mild hot flushes and sweatings	No	0	No
13	[illegible]3	162	66.5	11	Regular until 18 years old, then oligomenorrhoea	22	8	Normal	Moderate hot flushes and irritability	No	0	Yes (2 y)
14	19	160	45.2	15	Always slight oligomenorrhoea	18	12	Normal	Mild hot flushes and sweatings	No	0	No

20	170	5 μg	6.2	8.1	3.5	Normal	Normal	Positive	Stroma without any follicles	Atrophic	Combination prep. (6) Sequential prep. (12)
21	85	5 μg	6	5.8	6.5	Normal	Normal	Positive	Stroma with few primordial follicles	Slightly developed prol. phase	Sequential prep. (4)
22	100	6 μg	5.1	13.0	11.2	Normal	Normal	Positive	Stroma without any follicles	Atrophic	Sequential prep. (7) Oestrogens (12)
23	180	9 μg	5.6	7.3	7.2	Normal	Normal	Positive	Stroma without any follicles	Atrophic	Sequential prep. (23)
24	195	2 μg	4.3	10.8	4.2	Normal	Normal	Positive	Stroma with very few primordial and atretic follicles	Atrophic	Oestrogens (10)
25	80	7 μg	11.0	10.7	6.1	Normal	Normal	Positive	Stroma with numerous primordial + primary follicles	Slightly developed prol. phase	Sequential prep. (4)
26	235	8 μg	11.8	7.3	6.5	Normal	Normal	Positive	Stroma with primordial, primary and growing follicles	Atrophic	Combination prep. (2) Sequential prep. (12)

MATERIAL AND METHODS

During the period 1957 to 1971 539 patients with secondary amenorrhoea have been referred to and examined in our department. Twenty-six out of the 539 patients have met our criteria for the diagnosis of premature ovarian failure, which are (*a*) menarche at normal age, (*b*) normal or slightly underdeveloped secondary sexual characteristics, (*c*) secondary amenorrhoea before the age of 30 years, (*d*) increased excretion of gonadotrophins at repeated determinations, and (*e*) low excretion of oestrogens. Thus, the incidence of this syndrome among the patients referred because of secondary amenorrhoea was 4.8%. None of these patients had received any hormonal treatment during the last 6 months prior to the admission.

Table I shows the most important clinical data for the 26 patients. Ages ranged from 18 to 31 years, with an average of 26 years. The height was within the normal range in all patients, with an average of 162 cm, and the weight was within the normal range in all patients except for 2 (nos. 3 and 17) who were obese. Ages at menarche varied from 11 to 17 years, the average being 13 years. The menstrual pattern prior to the amenorrhoea is shown in the next column in Table I, 10 patients had regular periods, whereas 11 patients had regular periods for some years and then developed oligomenorrhoea before the onset of amenorrhoea and the last 5 patients had always had slight to severe oligomenorrhoea prior to the period of amenorrhoea. None of the patients had had an episode of secondary amenorrhoea previously, i.e. absent menstrual periods for 6 months or more. Ages at the time of the last period ranged from 13 to 29 years, with an average of 22 years. The duration of the secondary amenorrhoea prior to the first examination varied from 8 to 140 months, the average being 34 months.

It appears from Table I that secondary sexual characteristics were normally developed in all patients except for 3 (nos. 1, 7 and 22) who had already reached the menopause by the age of 13 to 16 years. It should also be noted that 12 patients did not have any symptoms of the climacteric at all, whereas the remaining 14 patients had at least some complaints, such as hot flushes, sweating, headaches, irritability and palpitations, but seldom as severe as found in post-menopausal women in the older age group. The family history revealed a tendency to an early menopause only in patient no. 10 whose mother and grandmother reached the menopause at an age of 34 and 32 years, respectively. Only 2 out of the 26 patients had been pregnant previously and both of them delivered a normal child at term. This pregnancy rate seems to be very low considering the average age of the patients. It should be noted, however, that besides the 2 patients, who had been pregnant previously and who did not complain of sterility, only 6 patients (nos. 2, 6, 13, 15, 21 and 25) had tried to conceive before the onset of amenorrhoea, and none of these for more than 2 years. The question of whether fertility is significantly decreased prior to the period of amenorrhoea is therefore still unsolved.

All patients were admitted to hospital for endocrine evaluation, including determinations of the urinary excretion of total gonadotrophins (9), total oestrogens bio-

Table II *Hormonal status and treatment of 20 patients with premature ovarian failure*

Patient no	Total gonado-trophins (MUU/day)	Total oestrogens (MUU/day or µg day)	17 KS (mg/day)	17 KGS (mg/day)	PBI (µg/100 ml)	X-ray of sella turcica	Fields of vision	X chromatin	Ovarian biopsy	Endometrial biopsy	Substitution therapy (months)
1	280	20 MUU	4.6	6.8	5.5	Normal	Normal	Positive	Stroma with very few atretic follicles	—	Sequential prep. (34)
2	145	20–200 MUU	8.2	6.7	5.7	Normal	Normal	Positive	—	Atrophic	No
3	270	20 MUU	10.2	10.9	7.0	Normal	Normal	Positive	Stroma without any follicles	Atrophic	Sequential prep (7)
4	225	20–200 MUU	4.7	7.6	6.8	Normal	Normal	Positive	—	Atrophic	No
5	170	20–200 MUU	9.2	7.9	6.1	Normal	Normal	Positive	Stroma with a few primordial follicles	Atrophic	Oestrogens (9)
6	210	20–200 MUU	8.9	7.4	5.9	Normal	Normal	Positive	—	Slightly developed prol. phase	Oestrogens (6)
7	140	20 MUU	8.4	5.8	5.4	Normal	Normal	Positive	Stroma without any follicles	Atrophic	No
8	360	20–200 MUU	9.6	13.1	6.0	Normal	Normal	Positive	—	Slightly developed prol. phase	Combination prep. (6) Sequential prep (11)
9	185	20 MUU	5.3	7.0	5.1	Normal	Normal	Positive	—	Atrophic	Combination prep. (9) Oestrogens (51)
10	95	20 MU	6.9	8.7	5.6	Normal	Normal	Positive	—	Atrophic	Oestrogens (4)
11	200	20 MUU	12.1	8.4	5.7	Normal	Normal	Positive	Stroma without any follicles	Atrophic	Sequential prep. (7)
12	280	20–200 MUU	5.7	8.3	4.6	Normal	Normal	Positive	—	Slightly developed prol. phase	No
13	275	20 MU	7.8	6.5	5.3	Normal	Normal	Positive	—	Atrophic	Oestrogens (12)
14	[illegible]	20–200 MU	6.5	4.6	3.6	Normal	Normal	Positive	—	Atrophic	No
15	210	20 MUU	11.1	8.8	5.4	Normal	Normal	Positive	Stroma with a few atretic follicles	Atrophic	No
16	205	20 MUU	13.3	8.5	5.2	Normal	Normal	Positive	—	Atrophic	No
17	90	16 µg	12.2	7.2	—	Normal	Normal	Positive	Stroma with very few atretic follicles	Atrophic	Sequential prep. (8)
18	100	3 µg	11.4	12.8	—	Normal	Normal	Positive	—	Atrophic	No
19	170	4 µg	8.7	7.6	—	Normal	Normal	Positive	Stroma without any follicles	—	Sequential prep.



Fig. 3. Ovarian biopsy from 26-year-old woman with premature ovarian failure (patient 26). One primordial follicle and one growing follicle, which has not yet reached the antrum stage, are seen in the normal ovarian stroma (285).

total thyroidectomy. This operation did not change her pituitary-gonadal function. The excretion of total gonadotrophins remained significantly increased and the secondary amenorrhoea persisted.

In all cases the radiological examination revealed a normal sella turcica, and the fields of vision were normal. All patients were X-chromatin positive.

Table II shows also the development and phase of the endometrium obtained by biopsy. Nineteen out of the 26 patients showed an atrophic endometrium, while 5 patients showed poorly developed endometrium in the proliferative phase. In the last 2 patients an endometrial biopsy was not performed.

Thirteen out of the 15 patients, in whom ovarian biopsies were obtained, showed on histological examination a normal ovarian stroma without, or with very few primordial and atretic follicles. Fig. 1 shows a typical case. This histological picture is very similar to that characteristic of the senescent ovary (Fig. 2). In the last 2 patients (nos. 25 and 26), however, the histological appearance of the ovary was quite different. In these cases the examination revealed a normal stroma with a morphologically normal ovarian follicular apparatus including a considerable number of primordial and primary follicles and even a few growing follicles (Fig. 3). The case histories of these 2 patients have previously been reported in more detail (19). It should be noted that the histological picture in these cases is very similar to that characteristic of the unstimulated ovary seen in patients with hypogonadotrophic amenorrhoea (Fig. 4).



Fig. 4. Ovarian biopsy from 23-year-old woman with untreated hypogonadotrophic primary amenorrhoea. Several primordial follicles and one growing follicle, which has not yet reached the antrum stage, are seen in the normal ovarian stroma (285).



Fig 1 Ovarian biopsy from an 18-year-old girl with premature ovarian failure (patient 1). Only few atretic follicles are seen in the stroma (×85).

logically or chemically (4), 17-ketosteroids (23), 17-ketogenic steroids (10), and the concentration of protein-bound iodine (PBI) in serum. In addition, an endometrial biopsy was performed in all patients except for 2 (nos. 1 and 19) and, in order to exclude a hypophyseal tumour radiological examination of the sella turcica was carried out and the fields of vision determined. An examination for X-chromatin was carried out in all cases and finally representative ovarian biopsies were obtained by laparotomy from 15 out of the 26 patients.

FINDINGS

Table II shows the hormonal status of the 26 patients investigated The values of total gonadotrophins are means of 3 or more determinations distributed over a period of several months, whereas all the other values given in this table are means of 2 determinations. The excretion of total gonadotrophins was determined biologically and expressed in mouse uterine units/day (MUU/day). The excretion of gonadotrophins was constantly increased in all patients, the values ranging from 80 to 360 MUU/day with an average of 195 MUU/day

Before 1969 the excretion of total oestrogens was determined biologically and expressed in mouse uterine units/day (MUU/day), but since then the excretion of oestrogens was determined by the method of Brown et al. (4). It appears from the table that the oestrogen excretion was very low in the majority of the patients.

The excretion of both 17 ketosteroids (17 KS) and 17-ketogenic steroids (17 KGS) was within the normal range in all 26 patients The concentration of PBI in serum was also within the normal range in all patients, in whom it was determined, except for patient no. 22 who had a toxic goitre and who later on underwent a sub-

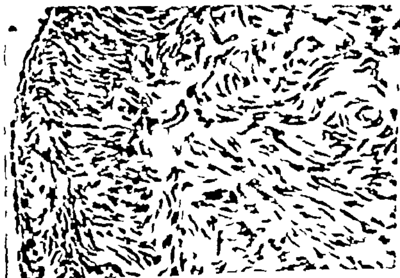


Fig Ovarian biopsy from a 53 year-old postmenopausal woman. Only very few atretic follicles are seen in the stroma (×85).



Fig 3 Ovarian biopsy from 26-year-old woman with premature ovarian failure (patient 26). One primordial follicle and one growing follicle, which has not yet reached the antrum stage, are seen in the normal ovarian stroma (285).

total thyroidectomy This operation did not change her pituitary-gonadal function. The excretion of total gonadotrophins remained significantly increased and the secondary amenorrhoea persisted

In all cases the radiological examination revealed a normal sella turcica, and the fields of vision were normal. All patients were X-chromatin positive

Table II shows also the development and phase of the endometrium obtained by biopsy Nineteen out of the 26 patients showed an atrophic endometrium, while 5 patients showed a poorly developed endometrium in the proliferative phase. In the last 2 patients an endometrial biopsy was not performed

Thirteen out of the 15 patients, in whom ovarian biopsies were obtained, showed on histological examination a normal ovarian stroma without, or with very few primordial and atretic follicles. Fig. 1 shows a typical case. This histological picture is very similar to that characteristic of the senescent ovary (Fig. 2). In the last 2 patients (nos 25 and 26), however the histological appearance of the ovary was quite different. In these cases the examination revealed a normal stroma with a morphologically normal ovarian follicular apparatus including a considerable number of primordial and primary follicles and even a few growing follicles (Fig. 3). The case histories of these 2 patients have previously been reported in more detail (19) It should be noted that the histological picture in these cases is very similar to that characteristic of the unstimulated ovary seen in patients with hypogonadotrophic amenorrhoea (Fig. 4)



Fig 4 Ovarian biopsy from 22-year-old woman with untreated hypogonadotrophic primary amenorrhoea. Several primordial follicles and one growing follicle, which has not yet reached the antrum stage, are seen in the normal ovarian stroma (285).



Fig 1 Ovarian biopsy from an 18-year-old girl with premature ovarian failure (patient 1). Only few atretic follicles are seen in the stroma (85)

logically or chemically (4), 17-ketosteroids (23), 17-ketogenic steroids (10), and the concentration of protein-bound iodine (PBI) in serum. In addition, an endometrial biopsy was performed in all patients except for 2 (nos. 1 and 19) and, in order to exclude hypophyseal tumour radiological examination of the sella turcica was carried out and the fields of vision determined. An examination for X-chromatin was carried out in all cases and finally representative ovarian biopsies were obtained by laparotomy from 15 out of the 26 patients.

FINDINGS

Table II shows the hormonal status of the 26 patients investigated. The values of total gonadotrophins are means of 3 or more determinations distributed over a period of several months, whereas all the other values given in this table are means of 2 determinations. The excretion of total gonadotrophins was determined biologically and expressed in mouse uterine units/day (MUU/day) The excretion of gonadotrophins was constantly increased in all patients, the values ranging from 80 to 360 MUU/day with an average of 195 MUU/day

Before 1969 the excretion of total oestrogens was determined biologically and expressed in mouse uterine units/day (MUU/day) but since then the excretion of oestrogens was determined by the method of Brown et al. (4). It appears from the table that the oestrogen excretion was very low in the majority of the patients.

The excretion of both 17 ketosteroids (17 KS) and 17 ketogenic steroids (17 KGS) was within the normal range in all 26 patients. The concentration of PBI in serum was also within the normal range in all patients in whom it was determined, except for patient no. 22 who had a toxic goitre and who later on underwent a sub-



Fig Ovarian biopsy from 53-year-old postmenopausal woman. Only very few atretic follicles are seen in the stroma (285)

acteristic of the postmenopausal ovary and is in good agreement with previous reports (2, 5 11, 14) More surprising was the finding, in the last 2 cases, of a normal ovarian stroma with a mor phologically normal follicular apparatus including numerous primordial and primary follicles and a few growing follicles. In spite of this picture, which is indistinguishable from that of the unstimulated ovary seen in patients with hypogona dotrophic amenorrhoea, it was not possible to stimulate the follicular development ven with very high doses of human gonadotrophins. Unfortunately we can not give any explanation for this phenomenon The possibility of an autoimmune reaction in the ovaries with formation of anti-gonadotrophins should be mentioned, but has not yet been proven.

It should be noted that only 14 out of the 26 patients in the present investigation had symptoms of the climacteric (hot flushes, sweating, headaches, irritability palpitations and tiredness), and even in these cases the nuisance was only of a mild to moderate degree. This finding confirms the observations of Baramki & Jones (2) Emperaire et al. (5), Keettel & Bradbury (11), Moraes-Ruehsen & Jones (14) and Zárate et al. (24). There is no doubt that all patients with such symptoms ought to receive substitution therapy The problem is, whether also patients without any symptoms at all should be treated in the same way prophylactically The strongest argument for this is that Millot & Daux (13) and Sznajderman & Oliver (21) have found that premature ovarian failure predisposes the patient to ischaemic heart disease They indicate an incidence of about seven times the average expectation of cardiac complication Furthermore, there seems to be a risk of severe osteoporosis because of the oestrogen deficiency These arguments, together with the unquestionable psychological effect of having regular menstrual periods just as other women in the same ge group makes it reasonable to re commend substitution therapy in all cases of premature ovarian f ilure Until lately we have used cyclical treatment either with sy thetic oestrogens or with a sequential preparation, but now prefer cyclical treatment with natural oestrogens

REFERENCES

1. Arias, A. R., La menopausia precoz y su tratamiento hormonal. Rev Med Chile 78 373, 1950.
2. Baramki, T A. & Jones, H. W Early premature menopause Amer J Obstet Gynec 96 990, 1966.
3. Björo, K. Amenorrhoea. A study with particular attention to the problems of ovarian failure. Acta Obstet Gynec Scand 45 (Suppl. 1) 69 1966
4. Brown, J B MacLeod, S. C., Macnaughtan, C., Smith, M. A. & Smyth, B: A rapid method for estimating oestrogens in urine using semi-automatic extractor. J Endocr 42 5 1968
5. Emperaire, J. C., Audebert, A. & Greenblatt, R. B. Premature ovarian failure. Report of seven cases. Amer J Obstet Gynec 108. 445, 1970.
6. Frankleton, M.: Classification and treatment of oligomenorrhoea and amenorrhoea. Acta Obstet Gynec Scand 39: 593, 1960.
7. Irvine, W J Premature menopause in autoimmune disease. Lancet 1 264 1969.
8. Jacobs, P A Baikie, A. G Brown, W M. C., MacGregor T N Maclean, N. & Harnden, D G.: Evidence for the existence of the human "super female Lancet 2. 423, 1959.
9. Johnsen, S. G A clinical routine-method for the quantitative determination of gonadotrophins in 24-hour urine specimens. Acta Endocr (Kbh) 28 69 1958
10. Jørgensen, M. On the determination of 17-ketogenic steroids in urine; excretion in normal subjects and after administration of corticotrophin. Acta Endocr (Kbh) 26. 424, 1957
11. Keettel, W C. & Bradbury J T Premature ovarian failure, permanent and temporary Amer J Obstet Gynec 89: 83, 1964.
12. Kinch, R. A. H., Plunkett, E. R., Smout, M. S. & Carr D. H., Primary ovarian failure. Amer J Obstet Gynec 91 630, 1965.
13. Millot, J & Daux, J L. Influence de la ménopause précoce, naturelle ou chirurgicale sur le déclenchement des coronarites Arch Mal Coeur 52 297 1959
14. Moraes-Ruehsen, M. de & Jones, G S Premature ovarian failure. Fertil Steril 18 440, 1967
15. Netter A. & Lebeaux, M. Ménopause précoce. Étude clinique, histologique et chromosomique de 16 cas, dont deux mosaïques. Relations avec les dysgénésies gonadiques et le syndrome de Stein. Gynéc Obstét (Paris) 66 249 1966
16. Perloff, W H. & Schneeberg, N G The premature climacterium. Amer Practitioner 8. 1955, 1957
17. Philip, J Sele, V & Trolle, D Secondary hypergonadotrophic amenorrhoea. Acta Obstet Gynec Scand 45 (Suppl. 9): 142, 1966
18. Sele, V & Starup, J Premature ovarian failure. Acta Obstet Gynec Scand 50 (Suppl. 9): 24, 1971
19. Starup, J Sele, V & Henriksen, B Amenorrhoea associated with increased production of gonadotrophins and a morphologically normal ovarian follicular apparatus Acta Endocr (Kbh) 66. 248, 1971
20. Serpanovsky B Estudio sobre el ciclo genital en

TREATMENT

The last column in Table II shows that substitution therapy has been started in 18 out of the 26 patients. It appears also that 3 patients previously have received cyclical treatment with a combination preparation but we now prefer cyclical treatment either with a sequential preparation or with oestrogens alone and this kind of treatment is now used in all cases. The effect on the symptoms of the climacteric has been excellent in all patients, and annoying side-effects have not been observed.

In the 2 patients (nos. 25 and 26) whose ovarian biopsies revealed a normal stroma with numerous morphologically normal follicles, we thought that there might be a possibility that stimulation therapy with human gonadotrophins would succeed. These 2 patients were therefore prior to the substitution therapy treated with very high doses of human menopausal gonadotrophin (HMG) in patient no 25 followed by a high dose of human chorionic gonadotrophin (HCG) but as previously reported (19) all attempts to stimulate follicular development and to achieve ovulation were unsuccessful.

DISCUSSION

The mechanism of follicular atresia in the physiological menopause is not as yet completely understood. Even more obscure is the aetiology of premature ovarian failure. Several different hypotheses have been proposed to explain the very early occurrence of atresia found in patients with this syndrome but the following 4 hypotheses seem to have gained most adherents. (*a*) a congenital and perhaps chromosomal anomaly resulting in a decreased ovarian stock of germinal cells (2 8 11 14 15) (*b*) a destruction of germinal cells in pre or postpubertal life caused by different agents, such as irradiation certain drugs and perhaps viruses (5 11 14) (*c*) a hypothalamic-pituitary dysfunction with maturation of an excessive number of follicles during each cycle resulting in massive follicular atresia (5 11) and (*d*) an autoimmune reaction in the ovary accelerating the atresia and making the remaining follicles less sensitive to stimulation with gonadotrophins (7). None of these hypotheses can however explain follicular atresia in all cases of premature ovarian failure and it seems likely that this syndrome can be caused by several different factors.

The typical menstrual history in patients with premature ovarian failure indicates a normal age at menarche with regular periods for various lengths of time followed either by amenorrhoea abruptly or by a period of oligomenorrhoea before the onset of amenorrhoea (2, 5 11 14 18 24) It is generally agreed that the secondary sexual characteristics are normal in these patients, which indicates that they previously have had a normal secretion of hormones from the ovaries. In addition both the present and several other studies (6 11 18 24) include a small number of patients who have been pregnant which is the most reliable proof of previously normal function of the ovarian follicles.

The diagnosis of premature ovarian failure is a serious one to the patient and therefore it should be made with due caution On the other hand it is very important that a definitive diagnosis can be established as soon as possible so that these young women can plan their lives and cease their futile search for fertility The clinical diagnosis of premature ovarian failure is difficult to establish as the symptomatology with the exception of the possible occurrence of hot flushes, is similar to that of other forms of amenorrhoea Repeated determinations of the urinary excretion of gonadotrophins is probably the most helpful test in the differential diagnosis, but it is very important that these analyses are distributed over a reasonable period of time since the level of gonadotrophins may fluctuate considerably and, therefore may give misleading information in some instances (6 11 19) When there is still doubt about the diagnosis, Zárate et al. (4) recommend that an ovarian stimulation test with HCG should be performed but in our opinion this test will only be helpful in very few cases. We are just as Baramki & Jones ( ) Emperaire et al (5) and Moraes Ruehsen & Jones (14) convinced that ovarian biopsies should be obtained either by laparotomy or by laparoscopy in these cases since histological examination of the ovary is by far the most reliable way to establish the diagnosis

Ovarian biopsies were obtained in 15 patients in the present investigation In 13 cases histological examination revealed normal ovarian stroma without or with only a few primordial and atretic follicles This picture is very similar to that char

Acta Obstet Gynec Scand 52: 269–272, 1973

"TRIGGERING" OF OVULATION AFTER INFUSION OF SYNTHETIC LUTEINIZING HORMONE RELEASING FACTOR (LRF)

Ryosuke Nakano, Tadayoshi Mizuno, Fumikazu Kotsuji, Kazuaki Katayama, Motoo Washio and Shimpei Tojo

From the Department of Obstetrics and Gynecology (Head: Prof. Shimpei Tojo), Kobe University School of Medicine, Kobe, Japan

Abstract. Synthetic luteinizing hormone releasing factor (LRF) was infused intravenously for 6 hours into two women who had donor insemination. Supplemental LRF was injected subcutaneously after 6 hours continuous infusion. Ovulation was triggered by LRF infusion and two successful pregnancies were achieved. It is suggested that ovulation might be triggered by continuous intravenous infusion of synthetic LRF if follicular development of the ovary is sufficient. It is concluded that "triggering" of ovulation by continuous infusion of LRF might be convenient means for controlling the timing of ovulation in case of donor insemination.

The chemical structure of isolated porcine luteinizing hormone releasing factor (LRF) has been proved to be (Pyro) Glu-His-Trp-Ser-Gly-Leu-Arg-Pro-Gly-NH_2 (1, 6, 12, 13) and the decapeptide corresponding to this structure has been synthesized (7). This synthetic LRF when administered as a rapid subcutaneous or intravenous injection, has been shown to be effective in increasing both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in the blood of both men and women (2, 4, 9, 14). This rapid method of injection, however, failed to induce ovulation (3). Since serum LH and FSH levels are elevated at least 12 hours in the normal ovulatory phase, a more prolonged gonadotrophin stimulation would be required to induce ovulation. For this purpose, a continuous 6 hours intravenous infusion of synthetic LRF was attempted in two women who request donor insemination, in order to synchronize the timing of ovulation and donor insemination.

The present paper reports the results of a continuous infusion of LRF into two women who had donor insemination. The ensuing pregnancies conclusively established the occurrence of ovulation.

MATERIALS AND METHODS

Toxicity studies of synthetic LRF have been performed and no serious side effects have been noted in any of the subjects given synthetic LRF (9).

Clinical subjects

Two normal ovulating women were given synthetic LRF in order to trigger ovulation in the preovulatory phase. These two women requested donor insemination and insemination was performed before continuous infusion of LRF. Donor insemination was tried only once in the preovulatory phase and the day selected for insemination was based upon: (a) knowledge of the length of 5 or more of the most recent, consecutive menstrual cycles, (b) daily basal body temperature readings and (c) findings of vaginal and cervical mucus smears. Immediately after insemination, synthetic LRF was infused according to the following schedule.

LRF administration

Synthetic LRF used in this study was generously supplied by Mochida Pharmaceutical Company, Tokyo, Japan.

In terms of the dosage of LRF employed in our previous clinical investigations (7), 600 μg of synthetic LRF was infused for 6 hours, supplemented with subcutaneous injection of 400 μg of LRF at 6 hours. Blood was sampled at zero time, ½, 1, 3, 6 and 10 hours for determination of serum LH and FSH concentration.

Serum LH and FSH levels were measured in duplicate by double antibody radioimmunoassay according to the method of Odell et al. (11) and Midgley (8) with modifications. Both the second international reference preparation of human menopausal gonadotrophin (2nd IRP-HMG) and LER 907 were used to obtain standard curves. The average relative potencies of these preparations were 304 IU of 2nd IRP-HMG/mg LER 907 for LH and 49 IU of 2nd IRP-HMG/mg LER 907 for FSH. The results of this study were expressed as mIU of 2nd IRP-HMG/ml serum.

un grupo de 700 mujeres. Prensa Med Argent *8* 511 1941
21 Smajderman, M. & Oliver M F Spontaneous premature menopause ischemic heart-disease and serum-lipids. Lancet *1* 96., 1963
2. Tisserand Perrier M Recherches sur la ménopause études de statistique comparative Gynéc Obstét (Paris) *5* 43 1953
23 Vestergaard, P Rapid micro-modification of the Zimmermann/Callow procedure for the determination of 17-ketosteroids in urine Acta Endocr (Kbh) *8* 193 1951
4 Zárate A., Karchmer S Gómez, E. & Castelazo-Ayala, L. Premature menopause A clinical, histological, and cytogenetic study Amer J Obstet Gynec *106* 110, 1970.

Submitted for publication Oct. 30, 197

Jørgen Starup
Abrinken 55
2830 Virum
Denmark

30 hours. The magnitude of the response of the serum LH and FSH was similar to that in the first case (Fig. 3).

Basal body temperature rose on the following day and the immunological pregnancy test was positive month later (Fig. 4).

No serious side effects (change in blood pressure, pulse and respiration) were noted, but, dizziness and nausea were noted in the second subject.

DISCUSSION

The recent elucidation of the structure and synthesis of luteinizing hormone releasing factor (LRF) (1 6, 7 12, 13) made it possible to perform clinical investigations. Synthetic LRF has been shown to be active in stimulating the secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in both men and women (2, 4 9 14) and has a diagnostic value for the evaluation of pituitary gonadotrophin reserve (9).

Another way of demonstrating hormonal response to LRF from target organ would be to induce ovulation. It has been reported that the rapid intravenous injection of LRF failed to cause ovulation in 4 women (3). Since serum LH is increased for at least 12 hours in the normal ovulatory phase it was assumed that a more prolonged gonadotropic stimulus would be required to produce ovulation. According to the report of Kastin et al. (5), LRF of porcine origin was infused intravenously for 24 hours into a woman with secondary amenorrhoea who was pretreated with human menopausal gonadotrophin (HMG-Pergonal). Supplemental LRF was injected intravenously *3 8 and 24 hours* during the infusion. Ovulation, indicated by the marked rise in urinary pregnanediol levels, was confirmed by pregnancy. This is the sole report of successful pregnancy after LRF administration.

In this series, two successful conceptions were achieved after donor insemination and a continuous infusion of synthetic LRF. The results suggest that ovulation might result from LRF infusion. Although a causal relationship between LRF and ovulation is not proved, it is possible to assume that ovulation was triggered by a continuous infusion of LRF. Although rising urinary pregnanediol levels in these two women together with the basal body temperature indicated that ovulation followed the infusion of LRF the best proof of ovulation in the human being is subsequent pregnancy. Their pregnancies constituted final proof of induced ovulation.

It is possible that ovulation and subsequent pregnancy were not caused by the infusion of LRF. Before this study however it was quite difficult to synchronize the timing of ovulation and donor insemination because of their menstrual irregularity. The results of the present investigation suggest that a continuous intravenous infusion of synthetic LRF produces a marked surge of serum LH and FSH to trigger ovulation, if follicular development of the ovary is sufficient. Therefore it might be concluded that the "triggering" of ovulation by LRF administration is a convenient way of controlling the timing of ovulation.

ACKNOWLEDGEMENT

We wish to acknowledge the National Pituitary Agency and the Endocrinology Study Section of the National Institute of Arthritis and Metabolic Diseases for the generous supply of materials for radioimmunoassay of human pituitary gonadotrophins.

The second international reference preparation of human menopausal gonadotrophin was obtained from Dr R. Bangham of the National Institute for Medical Research, London.

REFERENCES

1. Baba, Y. Matsuo, H. & Schally A. V. Structure of porcine LH- and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analysis. Biochem Biophys Res Commun 44 459 1971
2. Gual, C., Kastin, A. J. Midgley A. R., J. & Flores, F. Administration of LH-releasing hormone as clinical test of pituitary function. The fifty-third meeting of the Endocrine Society (Vol. 88), A 128, 1971.
3. Kastin, A. J. Schally A. V. Gual, C., Midgley A. R. J. Bowers, C. Y. & Gomez-Perez, F. Administration of LH-releasing hormone to selected human subjects. Amer J Obstet Gynec 108 177 1970
4. Kastin, A. J. Schally A. V. Gual, C., Midgley A. R., J. Miller M. C. & Cabeza, A. Dose-response relationship of LH to LH-releasing hormone in man. J Clin Invest 50 1551, 1971
5. Kastin, A. J. Zarate, A., Midgley A. R. Jr, Canales, E. S. & Schally A. V. Ovulation confirmed by pregnancy after infusion of porcine LH-RH. J Clin Endocr 33 [illegible], 1971
6. Matsuo, H., Baba, Y. Nair R. M. G., Arimura, A. & Schally A. V. Structure of the porcine LH- and

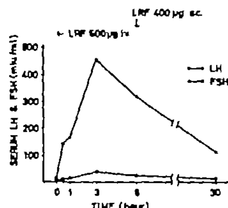


Fig 1 Serum LH and FSH levels during and after a continuous intravenous infusion of synthetic LRF (Case 1)

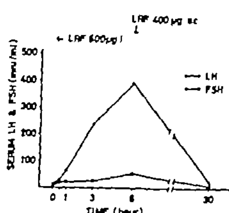


Fig 3 Serum LH and FSH levels during and after a continuous intravenous infusion of synthetic LRF (Case 2).

RESULTS

The first subject was a 44-year-old married woman who had received a donor insemination 4 times and had failed to conceive before this study Her basal body temperature record showed that the mean length of the follicular phase was 12.4 days but the menstrual cycle was quite irregular Donor insemination and LRF infusion were performed on Cycle Day 9

Serum LH and FSH response after intravenous infusion of synthetic LRF was shown in Fig. 1 A continuous 6 hours infusion of LRF resulted in elevation of serum LH and FSH to more than the normal levels for the ovulatory phase. The serum LH level rose from 30 minutes and reached a peak at 3 hours of LRF infusion Subsequently the serum LH level declined towards the baseline, but was still elevated at 30 hours. Although the magnitude of the response was small the serum FSH level rose from 30 minutes and a peak level occurred at 3 hours. The serum FSH level decreased gradually but remained elevated at 30 hours (Fig. 1)

After donor insemination and LRF administration urinary pregnandiol as well as basal body temperature rose and 24 days later the immunological pregnancy test was positive. Pregnancy has been subsequently confirmed (Fig. 2).

The second subject was a 25 year-old married female who had received a donor insemination 3 times previously and had failed to conceive Judging from her basal body temperature record, the menstrual cycle was irregular and the length of the follicular phase was 14 4 days. Donor insemination and LRF infusion were performed on Cycle Day 15

The serum LH and FSH levels after a continuous infusion of LRF are illustrated in Fig. 3. There was a clear rise of serum LH and FSH to more than the normal level for the ovulatory phase Serum LH and FSH levels began to rise from 30 minutes. The LH and FSH peak response occurred at 6 hours and remained elevated at

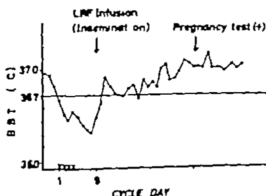


Fig 2 Basal body temperature record of the subject who had donor insemination and LRF infusion (Case 1).

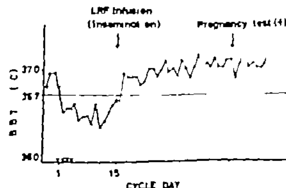


Fig 4 Basal body temperature record of the subject who had donor insemination and LRF infusion (Case 2)

30 hours. The magnitude of the response of the serum LH and FSH was similar to that in the first case (Fig. 3).

Basal body temperature rose on the following day and the immunological pregnancy test was positive a month later (Fig. 4).

No serious side effects (change in blood pressure, pulse and respiration) were noted, but, dizziness and nausea were noted in the second subject.

DISCUSSION

The recent elucidation of the structure and synthesis of luteinizing hormone releasing factor (LRF) (1 6, 7 12, 13) made it possible to perform clinical investigations. Synthetic LRF has been shown to be active in stimulating the secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in both men and women (2, 4 9 14) and has a diagnostic value for the evaluation of pituitary gonadotrophin reserve (9).

Another way of demonstrating hormonal response to LRF from a target organ would be to induce ovulation. It has been reported that the rapid intravenous injection of LRF failed to cause ovulation in 4 women (3). Since serum LH is increased for at least 12 hours in the normal ovulatory phase, it was assumed that a more prolonged gonadotropic stimulus would be required to produce ovulation. According to the report of Kastin et al (5), LRF of porcine origin was infused intravenously for 24 hours into a woman with secondary amenorrhoea who was pretreated with human menopausal gonadotrophin (HMG-Pergonal). Supplemental LRF was injected intravenously 18 and 24 hours during the infusion. Ovulation, indicated by the marked rise in urinary pregnandiol levels, was confirmed by pregnancy. This is the sole report of successful pregnancy after LRF administration.

In this series, two successful conceptions were achieved after donor insemination and a continuous infusion of synthetic LRF. The results suggest that ovulation might result from LRF infusion. Although a causal relationship between LRF and ovulation is not proved, it is possible to assume that ovulation was triggered by a continuous infusion of LRF. Although rising urinary pregnandiol levels in these two women together with the basal body temperature indicated that ovulation followed the infusion of LRF, the best proof of ovulation in the human being is subsequent pregnancy. Their pregnancies constituted final proof of induced ovulation.

It is possible that ovulation and subsequent pregnancy were not caused by the infusion of LRF. Before this study, however, it was quite difficult to synchronize the timing of ovulation and donor insemination because of their menstrual irregularity. The results of the present investigation suggest that a continuous intravenous infusion of synthetic LRF produces a marked surge of serum LH and FSH to trigger ovulation, if follicular development of the ovary is sufficient. Therefore it might be concluded that the "triggering" of ovulation by LRF administration is a convenient way of controlling the timing of ovulation.

ACKNOWLEDGEMENT

We wish to acknowledge the National Pituitary Agency and the Endocrinology Study Section of the National Institute of Arthritis and Metabolic Diseases for the generous supply of materials for radioimmunoassay of human pituitary gonadotrophins.

The second international reference preparation of human menopausal gonadotrophin was obtained from Dr R. Bangham of the National Institute for Medical Research, London.

REFERENCES

1. Baba, Y. Matsuo, H. & Schally, A. V. Structure of porcine LH- and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analyses. Biochem Biophys Res Commun *44* 459, 1971.
2. Gual, C., Kastin, A. J. Midgley, A. R., J. & Flores, F. Administration of LH-releasing hormone as clinical test of pituitary function. The fifty-third meeting of the Endocrine Society (Vol. 88), A-128, 1971.
3. Kastin, A. J. Schally, A. V. Gual, C., Midgley, A. R. J. Bowers, C. Y. & Gomez-Perez, F. Administration of LH-releasing hormone to selected human subjects. Amer J Obstet Gynec *108* 177, 1970.
4. Kastin, A. J. Schally, A. V. Gual, C., Midgley, A. R. J. Miller, M. C. & Cabeza, A. Dose-response relationship of LH to LH-releasing hormone in man, J Clin Invest 50. 1551, 1971.
5. Kastin, A. J. Zarate, A. Midgley, A. R., J. Canales, E. S. & Schally, A. V. Ovulation confirmed by pregnancy after infusion of porcine LH-RH. J Clin Endocr *33* 980, *1971*.
6. Matsuo, H. Baba, Y. Nair, R. M. G., Arimura, A. & Schally, A. V. Structure of the porcine LH- and

FSH releasing hormone I. The proposed amino acid sequence. Biochem Biophys Res Comm 43 1334 1971
7 Matsuo, H Arimura, A., Nair R. M. G & Schally A. V The synthesis of porcine LH and FSH releasing hormone by the solid phase method. Biochem Biophys Res Comm 45 654 1971
8. Midgley A. R., Jr Radioimmunoassay of human follicle-stimulating hormone J Clin Endocr 7 795 1967
9 Nakano, R., Kotsuji, F Mizuno, T Hashiba, N Washio, M. & Tojo, S. Response to luteinizing hormone releasing factor in normal subject and anovulatory patients. Acta Obstet Gynec Scand 5 171 1973.
10. Odell, W D Rayford, P L. & Ross, G. T Simplified, partially automated method for radioimmunoassay of human thyroid-stimulating, growth, luteinizing and follicle-stimulating hormones. J Lab Clin Med 70 973, 1967
11 Schally A. V Arimura, A., Kastin, A. J Matsuo, H Baba, Y Redding, T W Debeljuk, L., Nair R. M G & White W F A gonadotrophin releasing hormone: A single hypothalamic polypeptide hormone regulates the secretion of both LH and FSH Science 173 1036, 1971
12 Schally A. V Arimura, A., Baba, Y Nair, R. M. G. Matsuo, H., Redding, T W & Debeljuk, L. Isolation and properties of the FSH and LH-releasing hormone Biochem Biophys Res Comm 43 393, 1971
13 Schally A. V., Baba, Y., Arimura, A., Redding, T. W & White W F., Evidence for peptide nature of LH- and FSH-releasing hormones. Biochem Biophys Res Comm 4 50, 1971
14 Schally A. V Kastin, A. J & Arimura, A Hypothalamic FSH and LH regulating hormone Structure physiology and clinical studies. Fertil Steril 2 703, 1971

Submitted for publication Oct 4 197

Ryosuke Nakano
Dept. of Obstetrics & Gynecology
Kobe University School of Medicine
7 Kusunoki-Cho Ikuta-Ku
Kobe 650
Japan

Acta Obstet Gynec Scand 52. 273–275, 1973

PROSTAGLANDIN $F_{2\alpha}$ AND LABOUR

Hilary C. Brummer and Ian L. Craft

From the Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, London, England

Abstract. Serum $PGF_{2\alpha}$ levels during labour and the early post-partum period have been determined by radioimmunoassay. Random samples from 81 women showed that the highest levels are found in the late first stage of labour with lower levels in the second stage, at delivery and post-partum. The pattern in seven women who were followed through labour was similar to that seen in random samples and it was found that the change serum $PGF_{2\alpha}$ levels appears to be related to the stage of cervical dilatation.

It was first demonstrated by Karim (4) that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) could be detected in maternal venous blood during labour and that the levels at any given time seemed to be related to the contractile state of the uterus. More recently Hibbard (5) has shown that the highest levels are detected about 30 sec after the peak of contraction suggesting that $PGF_{2\alpha}$ arises as a result of the contractions. Studies carried out in this laboratory have shown that the highest levels are associated with the late first stage of labour when there is the greatest uterine activity (1). These studies have now been extended.

MATERIALS AND METHODS

Random samples of venous blood were taken from several women at various stages of labour. Seven other women were followed throughout labour. For this purpose, self-retaining cannula was inserted into an ante-cubital vein to assist the easy withdrawal of repeated samples. Where labour was induced by artificial rupture of the fetal membranes and Syntocinon infusion, the first sample was taken immediately prior to rupture of the membranes and the cannula was inserted at this time (5 cases). Subsequent samples were taken at regular intervals until delivery. Where the women were admitted in labour (2 cases), catheter was inserted during the early stages and the first sample withdrawn immediately and then at regular intervals until delivery. Where possible, further sample was also taken soon after delivery. 10 ml samples of blood were withdrawn and allowed to clot for 1 2 h at 4°C. The serum was then separated by centrifugation and the $PGF_{2\alpha}$ extracted and determined by radioimmunoassay as described previously (1).

RESULTS

Fig. 1 shows the mean $PGF_{2\alpha}$ levels in the sera of 58 women at various stages of labour and 23 women in the first week following delivery. The

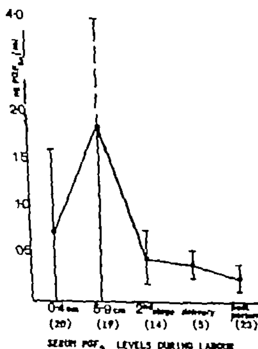


Fig. 1. Serum $PGF_{2\alpha}$ levels during labour. Values shown are mean ± standard deviation from the mean. The numbers in parenthesis denote the number of samples studied at each stage.

16 731183

FSH releasing hormone I. The proposed amino acid sequence Biochem Biophys Res Comm 43 1334 1971
7 Matsuo, H., Arimura, A., Nair R. M. G. & Schally A. V The synthesis of porcine LH and FSH releasing hormone by the solid phase method. Biochem Biophys Res Comm 45 654 1971
8. Midgley A. R., Jr Radioimmunoassay of human follicle-stimulating hormone. J Clin Endocr 27 795 1967
9 Nakano, R., Kotsuji, F Mizuno, T Hashiba, N Washio, M & Tojo, S., Response to luteinizing hormone releasing factor in normal subjects and anovulatory patients. Acta Obstet Gynec Scand 5 171 1973.
10. Odell, W D Rayford, P L. & Ross, G. T Simplified, partially automated method for radioimmunoassay of human thyroid-stimulating, growth, luteinizing and follicle-stimulating hormones. J Lab Clin Med 70 973, 1967
11 Schally A. V Arimura, A., Kastin, A. J Matsuo, H., Baba, Y Redding, T W Debeljuk, L., Nair R. M G. & White W F., A gonadotrophin releasing hormone: A single hypothalamic polypeptide hormone regulates the secretion of both LH and FSH Science 173 1036, 1971
1. Schally A. V Arimura, A., Baba, Y Nair R.M.G Matsuo, H., Redding, T W & Debeljuk, L. Isolation and properties of the FSH- and LH-releasing hormone Biochem Biophys Res Comm 43 393, 1971
13 Schally A. V Baba, Y Arimura, A., Redding, T.W & White, W F Evidence for peptide nature of LH- and FSH-releasing hormones. Biochem Biophys Res Comm 4 50, 1971
14 Schally A. V Kastin, A. J & Arimura, A. Hypothalamic FSH and LH-regulating hormone: Structure, physiology and clinical studies. Fertil Steril .. 703, 1971

Submitted for publication Oct 24 1972

Ryosuke Nakano
Dept. of Obstetrics & Gynecology
Kobe University School of Medicine
7 Kusunoki-Cho, Ikuta-Ku
Kobe 650
Japan

Acta Obstet Gynec Scand 52: 273–275 1973

PROSTAGLANDIN $F_{2\alpha}$ AND LABOUR

Hilary C. Brummer and Ian L. Craft

From the Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, London, England

Abstract. Serum $PGF_{2\alpha}$ levels during labour and the early post-partum period have been determined by radio-immunoassay. Random samples from 81 women showed that the highest levels are found in the late first stage of labour, with lower levels in the second stage, at delivery and post-partum. The pattern in seven women who were followed through labour was similar to that seen in random samples and it was found that the change in serum $PGF_{2\alpha}$ levels appears to be related to the stage of cervical dilatation.

It was first demonstrated by Karim (4) that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) could be detected in maternal venous blood during labour and that the levels at any given time seemed to be related to the contractile state of the uterus. More recently Hibbard (5) has shown that the highest levels are detected about 30 sec after the peak of contraction suggesting that $PGF_{2\alpha}$ arises as a result of the contractions. Studies carried out in this laboratory have shown that the highest levels are associated with the late first stage of labour when there is the greatest uterine activity (1). These studies have now been extended.

MATERIALS AND METHODS

Random samples of venous blood were taken from several women at various stages of labour. Seven other women were followed throughout labour. For this purpose, a self-retaining cannula was inserted into an ante-cubital vein to assist the easy withdrawal of repeated samples. Where labour was induced by artificial rupture of the fetal membranes and Syntocinon infusion, the first sample was taken immediately prior to rupture of the membranes and the cannula was inserted at this time (5 cases). Subsequent samples were taken at regular intervals until delivery. Where the women were admitted in labour (2 cases), a catheter was inserted during the early stages and the first sample withdrawn immediately and then at regular intervals until delivery. Where possible, a further sample was also taken soon after delivery. 10 ml samples of blood were withdrawn and allowed to clot for 1–2 h at 4°C. The serum was then separated by centrifugation and the $PGF_{2\alpha}$ extracted and determined by radioimmunoassay as described previously (1).

18 T12345

RESULTS

Fig. 1 shows the mean $PGF_{2\alpha}$ levels in the sera of 58 women at various stages of labour and 23 women in the first week following delivery. The

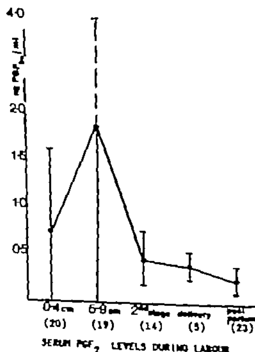


Fig. 1. Serum $PGF_{2\alpha}$ levels during labour. Values shown are mean ± standard deviation from the mean. The numbers in parentheses denote the number of samples studied at each stage.

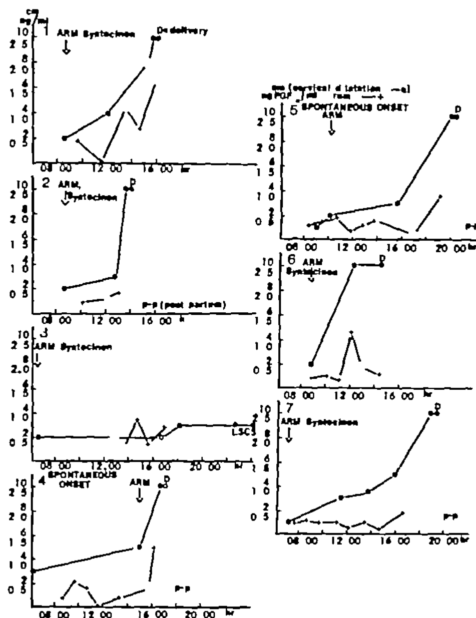


Fig 2 Serum PGF and cervical dilatation during labour Each graph represents one case. ARM indicates artificial rupture of the membranes.

highest levels were found at the time of greatest uterine activity during the late first stage of labour After full cervical dilatation had been reached, the mean level fell and remained low during the second stage at delivery and in the immediate post-partum period.

Seven other women were followed throughout their labour The serum $PGF_{2\alpha}$ levels and the stage of cervical dilatation for each of these women is shown in Fig. 2. Except for one value (case 1 at delivery) all the values were within one standard deviation from the mean found in the random samples. In nearly all cases the change in the $PGF_{2\alpha}$ levels reflected the stage of cervical dilatation as can be clearly seen from the figure. The same pattern was seen whether the labour was spontaneous in onset or induced.

DISCUSSION

Until recently it had been considered that the prostaglandins, and in particular $PGF_{2\alpha}$, might be responsible for the initiation and maintenance of uterine contractions in human labour (4) Recent research has suggested that rather than being the triggering mechanism these compounds arise as a result of the contractions (1 5). The results reported here lend further support to the latter theory

The fall which was usually seen in this investi-

gation during the second stage of labour and maintained at delivery is a rather different finding from that of Caldwell (2) and Collins (3), who reported peak levels at delivery although this was found in one patient in this study (case 1)

Further studies on individual patients with more frequent sampling, particularly during the later stages of labour are required to consolidate the relationship shown here, between $PGF_{2\alpha}$ cervical dilatation and uterine activity

ACKNOWLEDGEMENTS

HCB was supported by an Ortho Research Fellowship administered through the Royal College of Obstetricians and Gynaecologists. Special funds were provided by the SKF Foundation and Upjohn (UK) Ltd. Technical assistance was afforded by M C. Collins, Miss C. Parkinson, Miss J Weston and the Labour Ward Staff.

REFERENCES

1 Brosnan H. C., Serum $PGF_{2\alpha}$ levels during late pregnancy labour and the puerperium. Prostaglandins 2 185 1972.
2. Caldwell, B. V Burstein, S Brock, W A. & Speroff, L. Radioimmunoassay of the F prostaglandins. J Clin Endocr 33 171 1971.
3. Collins, W.: 1972 (personal communication).
4. Karim, S. M. M.. Appearance of prostaglandin $F_{2\alpha}$ in maternal venous blood during labour. Br Med J 4 618, 1968.
5 Hibbard, B. M.: 1972 (personal communication).

Submitted for publication Nov 28, 1972

Ian Craft
Institute of Obstetrics and Gynaecology
University of London
Queen Charlotte's Maternity Hospital
Goldhawk Road
London W6
England

ANNOUNCEMENTS

The Mammalian Fetus—Comparative Biology and Methodology

A symposium will be held on December 3–4 1973 at Wayne State University School of Medicine Detroit, Michigan, to dedicate C.S. Mott Center for Human Growth and Development. The symposium will cover comparative aspects of endocrinology growth, differentiation, neurobiology behavior metabolism, incompatibility mortality and morbidity of the mammalian fetus perinatal physiology detection of biochemical and chromosomal disorders and fetal retardation effects of maternal undernutrition, disease drugs and vaccination on fetal development and animal models for research. For further information write Dr E. S. E. Hafez, Department of Gynecology-Obstetrics, 550 E. Canfield, Detroit, Michigan 48201

Transport, Survival and Fertilizing Ability of Spermatozoa

A symposium organized by E. S. E. Hafez and C. Thibault, sponsored by INRA and INSERM of France will be held on November 4–7 1973 in Nouzilly near Tours, France. The symposium will cover comparative physiology biochemistry and biophysics of the male reproductive fluids and the epididymis, transport, survival and maturation of amphibian, reptilian, avian and mammalian sperm in the female reproductive tract clinical aspects of sperm transport in relation to regulation of human fertility and estrous synchronization in domestic animals with special emphasis to similarities and dissimilarities among man, nonhuman primates and laboratory and farm animals. Registration limited to 60 participants, should be made to Professor C. Thibault, Station de Recherches de Physiologie Animale, INRA, 78350 Jouy-en-Josas, France

First European Congress on Thermography

The First European Congress on Thermography will be held on June 17–20, 1974 Address: Organization Bureau, Amsterdam NV P O Box 7?05 Amsterdam, The Netherlands.

VIII World Congress on Fertility and Sterility

The VIIIth World Congress on Fertility and Sterility will be held in Buenos Aires, on November 3–9 1974

The official languages will be: Spanish, English, French and German, and there will be simultaneous interpretation at the scientific sessions.

The scientific programme is based on the following nine official topics.

1 Immunology aspects of reproduction.

2. New developments in the neuroendocrinology of reproduction.

3 Fertilization and early embryonic development in vivo and in vitro including genetic and epigenetic factors to the early embryonic development.

4 New developments in fertility control.

5 Male fertility

6. Recent progress.

7 Mechanism of hormone actions.

8. Psychology and social approaches to human reproduction including acceptability of fertility regulation methods.

9 New technical developments in therapeutic and diagnostic procedures.

A programme of free communications is open to members of Societies belonging to the International Federation of Fertility Societies (IFFS).

The Congress also includes a wide social and tourist programme which will help the participants and associates to enjoy what Buenos Aires has to offer in the way of interest and entertainment, as well as to see many places of beauty of the interior The Argentine Republic boasts the most beatiful scenery imaginable from snow capped mountains, noble forests, mirrorlike lakes, fashionable seaside resorts, to its vast pampas.

The officers of the Organizing Committee invite all colleagues to attend this important meeting.

All mail should be addressed to the Congress Secretariat as follows. VIII World Congress on Fertility and Sterility Avenida Roque Sáenz Peña 1110-2° piso, Buenos Aires, Argentine.

Acta Obstet Gynec Scand 52: 277–287 1973

NEW INSTRUMENTS

A NEW MECHANICAL INSTRUMENT FOR THE MEASUREMENT OF FIBRO-ELASTICITY WITH SPECIAL REFERENCE TO ITS USE IN THE ASSESSMENT OF THE CONSISTENCY OF THE UTERINE CERVIX

Trygve Bakke

From the Department of Obstetrics and Gynaecology (Head: Professor O. Koller), University of Bergen School of Medicine, 5000 Bergen, Norway

Abstract. A mechanical instrument for the measurement of fibro-elasticity is described. It consists of mechanism for control of the force used, which enables the examiner to exert the same pressure on the material each time, and another mechanism for recording the fibro-elasticity. The applied force *K* can be varied and is calibrated by the use of balance. The fibro-elasticity is characterized by the angle between the walls of the impressed area recorded on the indicator mechanism. The instrument has been tested on foam rubber materials, on water-filled rubber balloons, and on the uterine cervix both in vivo and in vitro. The results show that within large ranges of and *K* to first approximation the following relationship is valid:

$$F = \frac{\;}{K}$$

where F is constant which on relative basis can be used to characterize the elastic properties of material. A brief discussion of the properties and the applicability of the instrument is given.

The consistency of the uterine cervix is of great interest from many points of view in gynecology and obstetrics. The cervix is made up of connective tissue with smooth muscle fibres, elastic fibres and other elements in the ground substance and squamous and columnar epithelium covering the cervix. To the palpating finger there are differences in consistency of the cervical tissue from one woman to another and it is reasonable to expect variation of the cervical consistency during the menstrual cycle and in different age groups. In pregnancy the uterine cervix is softer than in the non-pregnant state. The softening is pronounced when labour has started, and Calkins (1) divided the cervical consistency in labour into four groups: (i) consistency which is almost mushy such as when the lip is relaxed, (ii) consistency which is definitely firmer such as when the lip is closed, (iii) consistency similar to the ala of the nose, and (iv) the rare, definitely pathological cervix of cartilaginous firmness.

In pregnancy and especially during labour three different characteristics of the uterine cervix are of special interest: the length of the cervical canal, the diameter of the cervical canal and the consistency of the uterine cervix.

Koller (5), in a series of 169 cases performed routine examinations of the cervix before induction of labour by amniotomy. He made objective measurements of the length of the cervical canal. Friedman (4) described an instrumental method for the study of cervical dilatation in labour.

To the author's knowledge no instrument has previously been made for the measurement of the consistency of the uterine cervix. The instrument to be described in this paper was designed for this purpose, and the instrument represents a new method for measuring the fibro-elasticity of a material.

PHYSICAL PROPERTIES OF FIBRO-ELASTIC MATERIALS

In technical terminology the human uterine cervix may be classified as a complex visco-elastic material. The term visco-elastic (3) here refers to the presence of at least two different physical properties, namely plasticity and elasticity. When small ball is pressed into a visco-elastic material, the geometry of the impressed area is dependent on the property of the material and may be used to characterize the material.

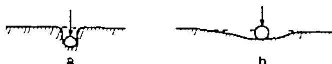


Fig 1 Plastic (*a*) and elastic (*b*) materials.

To illustrate these properties two examples are given in Figs. 1 *a* and *b* In Fig. 1 *a* the material has only plastic properties. The walls of the impression are vertical and the *depth* of the impression (dependent on the force used) will characterize the material. When the ball is removed, the impression lasts for some time without change.

In Fig. 1 *b* the material has elastic properties. When the ball is removed the impression disappears quickly In this case the depth of the impression is of less importance. Instead the *shape* of the impressed surface will characterize the material.

In practice a mixture of the two properties in varying proportions may be found in visco-elastic materials. It is therefore necessary to measure both the depth (plasticity) and the shape (elasticity) of the impression to characterize the material. These two characteristics may be called the *softness* and the *fibro-elasticity* of the material. In Fig. 2 *a* and *b* are shown the responses of two materials for which the fibro-elasticity (as characterized by the angle α) is the same, but the depth *d* of the impression is different. This occurs when two structures have different softness (characterized by the value of *d*) or when different pressures are used on the same structure. Hence, the way a material reacts upon impressed bodies depends on which of the two characteristics (softness and fibro-elasticity) is dominating in a material. For a soft material the geometry of the impressed body as well as the force used are of great importance. For an elastic material, on the other hand, the geometry is of less importance. When fibro-elastic properties dominate the relationship between the angle α and the applied force *K* is expected to be as shown in Fig. 3 Here the curve has an S-shape were α

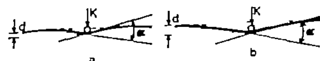


Fig 2 Fibro-elasticity (angle α), force (*K*) and depth of impression (*d*) in two situations (see text).



Fig 3 Theoretical curve for elastic materials. Fibro-elasticity expressed by α vs. force *K*.

increases rapidly with increasing *K* when the applied force is small and very high. In the intermediate region of *K* the slope of the curve is small.

METHODS OF INVESTIGATION

1 *Description of the instrument*

An instrument for measurements on the uterine cervix must have suitable dimensions for vaginal examinations and be constructed for horizontal use since the vagina is nearly horizontal during clinical examination of the cervix. It should not cause any discomfort or injury to the patient and must permit sterilizing procedures. The instrument must have one mechanism which enables the examiner to exert the same pressure from the instrument to the cervical tissue each time and it must be possible to calibrate this mechanism for different values of the applied force K. Finally the instrument must have a mechanism for recording the fibro-elasticity of the tissue.

The instrument (see Fig. 4 *a*) consists of a steel bar with a mechanism for control of the force used at one end and a mechanism for recording fibro-elasticity of the material being measured at the other end. The total weight of the instrument is 29 g, and the total length 251 mm.

In Fig. 4 *b* and is shown the mechanism for control of the applied force when the instrument is used. A steel tube slides on the steel bar. The steel tube has a

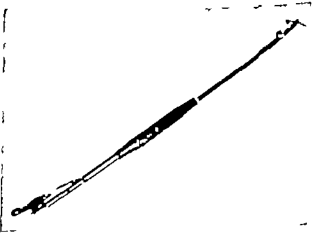


Fig 4 a. The instrument, with the indicator mechanism to the right and the mechanism for control of the applied force to the left on the picture.

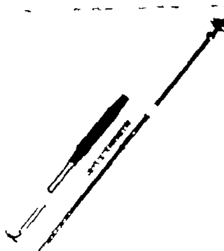


Fig. 4 b. Disassembled parts of the mechanism for control of force.

bearing at each end, and the tube itself serves as the grip of the instrument. Within this tube is helical spring. The tension of the spring is controlled by a calibrated stop mechanism, and to this is mounted a stop hook (see Fig. 4 *d*).

The indicator mechanism is mounted on the other end of the instrument (see Fig 4 and *f*). This end is formed like an Indian ink pen, and the "nib" opening is controlled by screw. A thin axis connects the ends of the two split blades, and two indicator wings rotate on this axis. The wings are formed as sectors of circle

Fig. 4 c. Mechanism for control of the applied force.





Fig. 4 *d*. Position of the stop hook at start of measurement (above) and after the desired force has been applied and the "click" released (below).

($r = 11$ mm) and are made of a 0.1 mm thick beryllium copper foil. On one of the wings is scale calibrated in "scale units" from 1 to 10, making total of 75 angle degrees.

Between and on each side of the wings are small discs of beryllium copper foil to ensure that the friction is kept at minimum.



Fig. 4 The indicator mechanism of the instrument.

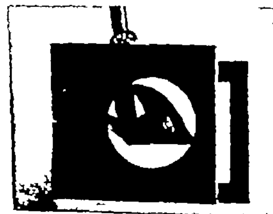


Fig. 4 *f*. Indicator end of instrument with scale under magnifying glass.

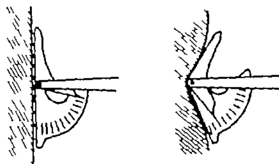


Fig 5 Position of indicator wings before and after measurement.

2. Operation of the Instrument

The experiments described in this paper were performed with the instrument in a horizontal position. When the instrument is held by the grip and the indicator end is pressed lightly against a material, the sliding tube moves toward the indicator end. The stop hook slips over the end of the tube into a small groove and a "click" of the stop hook is heard when the desired force has been applied (see Fig. 4 *d*)

When the indicator end of the instrument is pressed against a material, the indicator wings slide over one another (Fig. 5). The angle between them depends on the fibro-elasticity of the material in such a way that the angle decreases with increasing softness of the material. The reason for this is that the nearly ball-shaped indicator end of the instrument penetrates deeper into a soft than a hard material.

When the measurement is completed, the instrument is withdrawn, but the position of the indicator wings will not change due to friction between them, and the measured value can be read under a magnifying glass (Fig. 4 *f*).

3 Calibration of the Instrument

Fig. 6 shows the instrument and the equipment used for the calibration of the force control mechanism and the indicator mechanism.

The mechanism for control of the applied force was calibrated by pressing the indicator end of the instrument against a balance (Fig. 7) with the instrument in a horizontal position. The calibrated stop mechanism was adjusted so that the "click" of the stop hook (Fig. 4 *d*) was heard when the correct force was read on the balance.

The indicator mechanism was calibrated by a specially constructed instrument (Fig. 8 *a*). Each of the indicator wings was simultaneously pressed against two bronze lamella springs, one lamella spring for each indicator wing (Fig. 8 *b*). The distance between the lamella springs was kept at 18 mm in the experiments. Each lamella had a free ending measuring 20 mm 4 mm x 0.05 mm. When

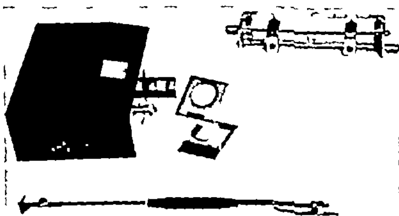


Fig 6 The instrument with equipment used for calibration.

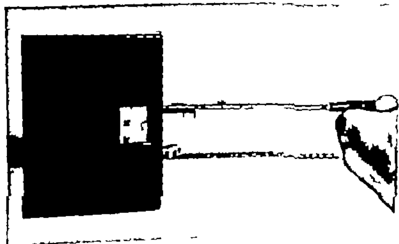


Fig 7 Calibration of mechanism for control of applied force.

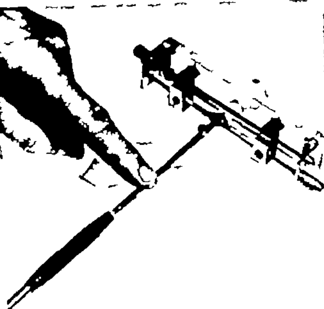


Fig. 8 a. Calibration of indicator mechanism in special instrument.

the end of the indicator mechanism had passed beyond the plane of the lamellae at distance of 6 mm it was stopped by fixed screw (see Fig. 8 *b*). During this procedure the indicator wings slid over one another because of the spring forces from the lamellae, and the friction between the indicator wings as adjusted so that the value of 8 scale units was read on the indicator scale hen the calibration was completed.

The mechanisms of the instrument were calibrated immediately before measurement as performed.

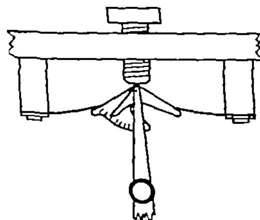


Fig. 8 b. Detail of instrument for calibration of indicator mechanism hen indicator end has touched the fixed screw and lamella springs have moved the indicator wings.

4. *Discussion of errors*

In the following 8 paragraphs possible errors in the measurements with the instrument are described and discussed.

1) The indicator wings move in opposite directions during measurement. In this way the movement of the wings is smoother and the deflection doubled. With magnifying glass it is not difficult to read the "scale units" to one decimal. The distance between the wings is 0.1 mm, and the error due to parallax therefore is insignificant. In the reading of the scale units following error is introduced.

$$\pm \Delta x_r \simeq \frac{1}{10} \text{ scale unit.}$$

2) All measurements were performed at room temperature and shortly after calibration of the instrument in the same room. Hence, errors due to temperature variations (Δx_t) can be ignored. For the same reason errors due to variations in humidity (Δx_h) are negligible.

3) The forces involved in operation of the instrument are subject to weight and inertia of the instrument and its parts, and to elastic spring forces. The resulting force is reset before each measurement as mentioned before (see Fig. 7). Adjustment of the mechanism for control of the applied force requires dexterity as well as patience. The error, ΔK_m of this examination has been found to be less than 1/ gram here 5 < < 10.

4) Hysteresis in the force control mechanism due to friction is also present. This introduces an error ΔK_h measured to be about 2 g hen 60 g force is used. The value of the relative error $\Delta K_h / K$ is probably independent of the force used and hence,

$$\Delta K_h = \frac{1}{30} K$$

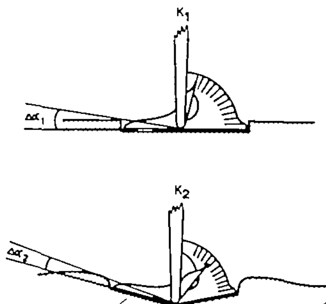


Fig 9 Influence of friction (ΔK_1) in the indicator mech*anism on reading when (above) a soft material is meas*ured by a small force K (Fig. 9 *a*) and (below) a stronger force K (Fig. 9 *b*).

5) Weight and inertia of the parts of the indicator mechanism and frictional resistance in these parts are also present during operation. This mechanism is therefore also calibrated before measurement (Fig. 8 *a* and *b*).

Every effort was made to reduce the error ΔK (and $\Delta\alpha_1$) due to friction between the indicator wings as much as possible. The small beryllium copper discs between and on each side of the wings have been useful, and the pressure on the split blades at the end of the instrument was kept at a minimum.

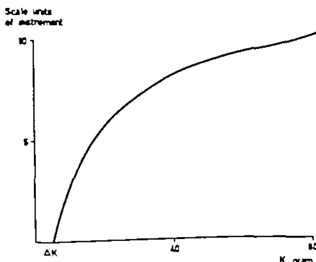


Fig 10 Lapse of curve if one material is measured with different forces K ΔK represents the friction (see text). elasticity of the material resulting in an error $\Delta\alpha$ in the

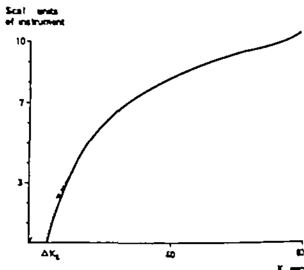


Fig 11 Measured curve solid line curve corrected" for friction broken line Values for $\Delta\alpha$ shaded area. Friction force· ΔK See text.

The friction between the indicator wings also has another influence on the measurements. As shown by Fig. 10 the curve does not start at zero but at some distance ΔK to the right on the horizontal line. Without this friction and excluding other effects the curve should start at zero. Fig. 9 *a* and *b* present tw schematic diagrams illustrating the response of soft material subjected to a small (K_1) and a strong (K_2) force. In Fig. 9 *a*, the friction force ΔK is greater than the fibro elasticity of the material resulting in an error $\Delta\alpha_1$ in the value of α and the indicator wings do not move at all ($\alpha_1 = 0$). In Fig. 9 *b* $K > \Delta K$ and $\Delta\alpha_2$ is smaller

The error $\Delta\alpha_1$ is highest when $K = \Delta K$ and decreases with increasing K Although ΔK is unknown one may make corrections graphically as illustrated in Fig. 11 The value of ΔK as suggested in the graph can be estimated as follows, since ΔK seems t be dependent on K.

(I) $\Delta K' = \Delta K - \frac{1}{2} K$ when $K < {}_2\Delta K$

where $\Delta K'$ is defined above and

(II) $\Delta K' = 0$ when $K \geq {}_2\Delta K$

The value of $\Delta\alpha_1$ will always be higher for softer than for harder materials. The friction force ΔK has been measured to be between 3 and 4 g when the indicator mechanism is calibrated to 8 scale units (see Fig. 8 and *b*).

When more than 3 scale units are read on the instrument ($\alpha > 20°$) the error due to friction may be ignored. Then the value f $\Delta\alpha/\alpha$ is equal t the relative error in the angle read on the instrument and $\Delta K/K$ is estimated from the relative errors in the adjustment and the reproducibility of the applied force

6) As pointed out by others (?) it is important not to stop or change direction of an instrument during measurements. The error $\Delta\alpha_{m}$, due to such ffects can be ignored in the present experiments.

7) Mechanical inaccuracies in any part of the instrument were avoided as much as possible when the instrument was made. A special mark on the grip of the instrument prevents the tube from rotating from one meas-

Table I. *Measurement of fibro-elasticity using different forces*

		Foam-rubber material A							Foam-rubber material B						
		Scale units of instrument				$\lambda = \alpha - \bar{\alpha}$			Scale units of instrument				$\lambda = \beta - \bar{\beta}$		
No.	K (g)	α_1	α_2	α_3	$\bar{\alpha}$	λ_1	λ_2	λ_3	β_1	β_2	β_3	$\bar{\beta}$	λ_1	λ_2	λ_3
1	3	0.9	0.4	0.7	0.67	0.23	−0.27	0.03	0.4	0.5	0.3	0.40	0.00	0.10	−0.10
2	5	1.6	1.5	1.9	1.67	−0.07	−0.17	0.23	0.9	1.2	1.0	1.03	−0.13	0.17	−0.03
3	8	3.1	3.0	3.2	3.10	0.00	−0.10	0.10	1.8	1.9	2.2	1.97	−0.17	−0.07	0.23
4	11	3.9	4.0	3.8	3.90	0.00	0.10	−0.10	2.4	2.2	2.3	2.30	0.10	−0.10	0.00
5	14	4.8	4.5	4.9	4.73	0.07	−0.23	0.17	2.2	2.5	2.4	2.37	−0.17	0.13	0.03
6	16	5.5	5.4	5.5	5.47	0.03	−0.07	0.03	3.4	3.5	3.4	3.43	−0.03	0.07	−0.03
7	21	7.0	7.0	6.9	6.97	0.03	0.03	−0.07	4.2	4.1	4.2	4.17	0.03	−0.07	0.03
8	26	7.8	8.0	7.5	7.77	0.03	0.23	−0.27	5.0	4.9	5.2	5.03	−0.03	−0.13	0.17
9	33	8.1	8.1	8.0	8.07	0.03	0.03	−0.07	5.1	5.8	5.6	5.50	−0.40	0.30	0.10
10	35	8.8	8.9	9.0	8.90	−0.10	0.00	0.10	6.6	6.8	6.6	6.67	−0.07	0.13	−0.07
11	38	8.9	9.0	9.0	8.97	−0.07	0.03	0.03	7.0	7.1	7.0	7.03	−0.03	0.07	−0.03
12	44	9.5	9.5	9.4	9.47	0.03	0.03	−0.07	7.4	7.2	7.1	7.23	0.17	−0.03	−0.13
13	48	9.7	9.5	9.6	9.60	0.10	−0.10	0.00	7.2	7.4	7.5	7.37	−0.17	0.03	0.13
14	50	9.6	9.7	9.7	9.67	−0.07	0.03	0.03	7.9	8.0	7.9	7.93	−0.03	0.07	−0.00
15	55	10.0	9.8	9.9	9.90	0.10	−0.10	0.00	7.7	8.0	8.0	7.90	−0.20	0.10	0.10
16	58	9.9	10.0	10.0	9.97	0.07	0.03	0.03	8.1	8.2	8.4	8.23	−0.13	−0.03	0.17
17	60	10.0	10.1	10.0	10.03	−0.03	0.07	−0.03	8.0	8.1	8.0	8.03	−0.03	0.07	−0.03
18	66	10.0	10.1	10.1	10.07	−0.07	0.03	0.03	8.6	8.8	8.9	8.77	−0.17	0.03	0.13
19	70	10.4	10.5	10.5	10.47	−0.03	0.07	−0.03	9.0	8.9	9.0	8.97	0.03	−0.07	0.03
20	73	10.4	10.5	10.5	10.47	0.03	0.07	−0.03	9.0	9.0	9.0	9.00	0.00	0.00	0.00
21	[illegible]	10.5	10.5	10.5	10.50	0.00	0.00	0.00	9.4	9.6	9.4	9.47	−0.07	0.13	−0.07

ment to another to avoid changes in the frictional force.

B) The instrument was handled with care and was not bent or deformed. Without such precaution the operating forces will change due to greater friction.

TEST EXPERIMENTS

The instrument was tested on two foam-rubber materials, a softer material A measuring 85 × 85 × 15 mm, and a harder material B measuring 80 × 80 × 5 mm. The measurements were done in the middle of the test pieces, which were held vertically.

It was also tested on a rubber balloon filled with 110 and [illegible] ml of water. The balloon was placed on a table and marked so that the same place was measured each time.

A complete uterus removed because of a benign disease was also a test object. Immediately after removal the uterus was placed on a table so that the cervix was easily reached with the instrument.

A series of measurements were also performed on uterine cervix in vivo. The anterior lip was used in the measurements of the two uteri, and the cervices were normal. In the measurements on the uterus in vivo only single readings for each force applied in the test series were taken since the measurements would otherwise be very time-consuming and annoying for the patient. All other measurements were performed three times at each force used in the series. The indicator mechanism was calibrated when a new force was used, and the force was measured by means of the balance described earlier (Fig. 7). As mentioned above, the measurements were done by horizontal application of the instrument.

TEST RESULTS

The results obtained in the measurements of the foam rubber materials, rubber balloon filled with water and the uterus both in vivo and in vitro are listed in Tables I–III and shown graphically in Figs. 12–15.

The curve (Fig. 12) represents the results obtained with the foam-rubber material A. Fig. 13 illustrates the results with the foam-rubber B of harder consistency. The curve for material A is steeper and reaches the more horizontal part earlier than the curve for material B. Both curves are approximately a straight line up to about $K = 20$ g and $K = 30$ g, respectively. The last portion of both curves shows a slight rising trend. It must be noted that the maximum reading of the instrument is 10.5 scale units.

Curve A (Fig. 14) represents the measurements of the rubber ballon filled with 110 ml of water. This curve is nearly a straight line up to $K = 20$ g. Curve B (Fig. 14), representing the balloon

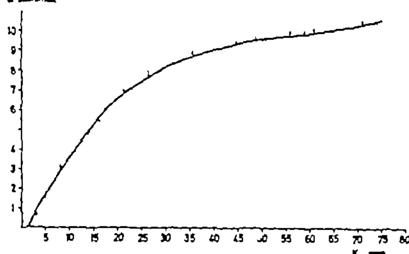


Fig. 12. Measurement of fibro-elasticity of material A. Vertical lines represent range and points the mean of actual observations.

filled with 180 ml of water has the same slope as the other curve and is almost a straight line up to K = 30 g. There is no pronounced horizontal part of these curves.

In Fig. 15 curve A represents the results obtained with the uterus in vitro. Measurements were not performed beyond 31 g since the uterus could be pushed during measurement when greater forces were applied. The curve is very nearly a straight line over the whole range.

The uterus in vivo is represented by curve B in Fig. 15. The cervix of this uterus was softer to the palpating finger than the cervix of the uterus in vitro. These differences are clearly demonstrated by the two curves in Fig. 15.

The measurements show that the maximum reading of the instrument is reached by application of smaller forces when the material is softer and that the rising and approximately straight part of the curve is shorter when soft materials are measured with increasing forces. When different materials are measured by the same force the highest value of α is found for the softest material.

The results of measurements show that over a wide range the following relationship between the angle α and the force K is valid.

$$\alpha = F \cdot K \quad (1)$$

where F is a constant which on a relative basis can be used to characterize the elastic properties of the material.

$$\text{Thus } F = \frac{\alpha}{K} \quad (2)$$

The fibro-elasticity expressed by F of the different test materials in this experiment may be calculated from the curves (Figs. 12–15) at for instance $K = 20$ g, and the results are shown in Table IV.

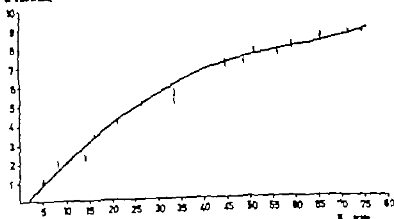


Fig. 13. Measurement of fibro-elasticity of material B. Vertical lines represent range and points the mean of actual observations.

Table II. Measurement of fibro-elasticity using different forces (rubber balloon filled with different amounts of water (A and B)

No.	Force K (g)	Rubber balloon filled with 110 ml of water (A)							Rubber balloon filled with 180 ml of water (B)						
		Scale units of instrument				$\lambda = \alpha - \bar{\alpha}$			Scale units of instrument				$\lambda = \beta - \bar{\beta}$		
		α_1	α_2	α_3	$\bar{\alpha}$	λ_1	λ_2	λ_3	β_1	β_2	β_3	$\bar{\beta}$	λ_1	λ_2	λ_3
1	4	0.5	1.0	0.9	0.80	−0.30	0.20	0.10							
2	7								0.6	0.5	0.5	0.53	0.07	−0.03	−0.03
3	8	2.8	2.3	2.3	2.53	0.27	−0.03	−0.23							
4	10	3.0	2.9	3.0	2.97	0.03	−0.07	0.03	1.1	1.0	1.3	1.13	−0.03	−0.13	0.17
5	15	4.3	4.1	3.9	4.10	0.20	0.00	−0.20	2.6	2.4	2.4	2.47	0.13	−0.07	−0.07
6	20	5.4	5.5	5.6	5.50	−0.10	0.00	0.10							
7	21								4.0	4.1	4.1	4.07	−0.07	0.03	0.03
8	24	6.0	5.8	5.9	5.90	0.10	−0.10	0.00							
9	25								4.5	4.8	4.8	4.70	−0.20	0.10	0.10
10	30								6.1	6.1	6.0	6.07	0.03	0.03	−0.07
11	31	7.8	7.3	7.2	7.43	0.37	−0.13	−0.23							
12	39	8.0	8.3	8.1	8.13	−0.13	0.17	−0.03	7.5	7.1	7.5	7.37	0.13	−0.27	0.13
13	45	9.0	9.1	9.0	9.03	0.03	0.07	−0.03	8.1	7.9	8.0	8.00	0.10	−0.10	0.00
14	49	10.1	9.9	10.0	10.00	0.10	−0.10	0.00							
15	54								9.2	8.9	9.0	9.03	0.17	−0.13	−0.03
16	59								9.5	9.6	9.6	9.57	−0.07	0.03	0.03
17	63								10.2	10.0	10.2	10.13	0.07	−0.13	0.07
18	68								10.5	10.5	10.5	10.50	0.00	0.00	0.00

The relative uncertainty of F is given by

$$\pm\frac{\Delta F}{F} = \left[\left(\frac{\Delta\alpha}{\alpha}\right)^2 + \left(\frac{\Delta K}{K}\right)^2\right]^{\frac{1}{2}} \qquad (3)$$

where $\Delta\alpha$ and ΔK are the total errors in α and K respectively.

Equation [2] is most reliable in the middle range of the curve since (*a*) friction disturbs the measurements when small forces are applied (see Figs. 10 and 11), and (*b*) for large angles the inclination of the impressed walls is too steep ($\alpha = 60-80°$). In the latter case other properties of the material in addition to those we are interested in determine the geometry and the angle. It therefore appears to be best to perform the experiments in the region of 3 to 8 scale units.

CONCLUDING REMARKS

Different improvements of the instrument could perhaps reduce some of the errors described. The split blades of the indicator end could be longer

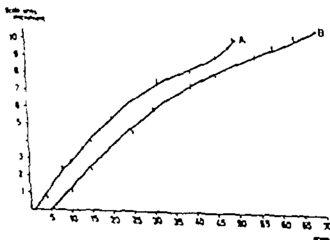


Fig. 14. Measurement of fibro-elasticity of rubber balloon filled with different amounts of water (*A* and *B*). Vertical lines represent range and points the mean of actual observations.

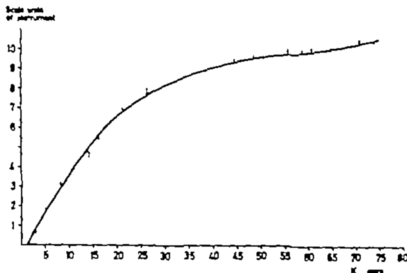


Fig 12 Measurement of fibro-elasticity of material A. Vertical lines represent range and points the mean of actual observations.

filled with 180 ml of water has the same slope as the other curve and is almost a straight line up to $K = 30$ g. There is no pronounced horizontal part of these curves.

In Fig. 15 curve A represents the results obtained with the uterus in vitro Measurements were not performed beyond 31 g since the uterus could be pushed during measurement when greater forces were applied. The curve is very nearly a straight line over the whole range.

The uterus in vivo is represented by curve B in Fig. 15 The cervix of this uterus was softer to the palpating finger than the cervix of the uterus in vitro These differences are clearly demonstrated by the two curves in Fig. 15

The measurements show that the maximum reading of the instrument is reached by application of smaller forces when the material is softer and that the rising and approximately straight part of the curve is shorter when soft materials are measured with increasing forces. When different materials are measured by the same force, the highest value of α is found for the softest material.

The results of measurements show that over a wide range the following relationship between the angle α and the force K is valid

$$\alpha = F \cdot K \quad (1)$$

where F is a constant which on a relative basis can be used to characterize the elastic properties of the material.

$$\text{Thus } F = \frac{\alpha}{K} \quad (2)$$

The fibro-elasticity expressed by F of the different test materials in this experiment may be calculated from the curves (Figs. 12–15) at for instance $K = 20$ g and the results are shown in Table IV

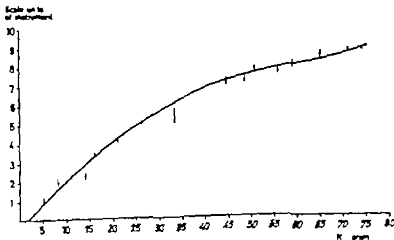


Fig 13 Measurement of fibro-elasticity of material B Vertical lines represent range and points the mean of actual observations.

vitro have been measured in a series of experiments. These measurements indicate that the fibro-elastic properties are important in the cervix, since the test results with the uteri closely resemble the results obtained for other fibro-elastic materials. The relative constant F is a useful index for the elastic property of the tissue, and the highest value is found in the softest material. Another series of experiments have therefore been carried out where constant forces were exerted from the instrument to the uterine cervix in different stages of pregnancy and the menstrual cycle. The results of the latter investigation will be given in another report.

REFERENCES

1. Calkins, L. A., Normal Labor. A Monograph in American Lectures in Gynecology and Obstetrics (ed. E. C. Hamblen). Charles C. Thomas, Springfield, Illinois, 1955
2. Donnell, L. H. & Savage, W. T. *In* Handbook of Experimental Stress Analysis (ed. M. Hetényi), p. 72. John Wiley & Sons, Inc., New York. Chapman & Hall, Limited, London, 1950.
3. Flügge, W. Viscoelasticity. Blaisdell Publishing company. Waltham, Mass., – Toronto – London, 1967
4. Friedman, E. A., Cervimetry: An objective method for the study of cervical dilatation in labor. Amer J Obstet Gynec *71* 1189. 1956.
5. Koller, O., Criteria for safe induction of labour by amniotomy. Acta Obstet Gynec Scand *34* 428, 1955.

Submitted for publication November 10, 1972

Trygve Bakke
Kvinneklinikken
5000 Bergen
Norway

Table III *Measurement of cervical consistency of non-pregnant uteri using different forces*

No.	Force K (g)	Uterine cervix in vitro (A): Scale units of instrument α_1	α_0	α	$\bar{\alpha}$	$\lambda = \alpha - \bar{\alpha}$: λ_1	λ_2	λ_3	Uterine cervix in vivo (B): Scale units of instrument (single measurement)
1	6	0.1	0.0	0.2	0.10	0.00	−0.10	0.10	1.8
2	10	1.4	0.9	1.0	1.10	0.30	−0.20	−0.10	2.4
3	14	1.6	2.1	2.0	1.90	−0.30	0.20	0.10	
4	16								3.5
5	20	3.2	3.3	3.2	3.23	−0.03	0.07	−0.03	
6	25	4.1	4.0	4.0	4.03	0.07	−0.03	−0.03	6.0
7	28								6.5
8	31	5.1	5.0	5.0	5.03	0.07	−0.03	−0.03	
9	36								7.2
10	42								7.0
11	47								8.1
12	55								9.0
13	62								9.2
14	66								9.8
15	70								10.5

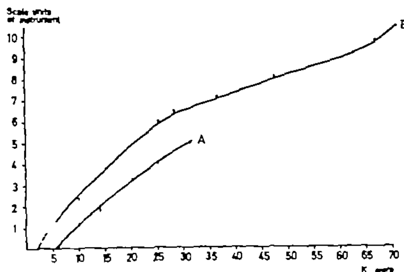


Fig 15 Measurement of cervical consistency of non-pregnant uteri. (A) Uterus in vitro, vertical lines represent range and points the mean of actual observations. (B) uterus in vivo, points represent single observations at each force.

and thinner whereby the friction between the indicator wings might be reduced and the adjustment be easier to perform. One could further more reduce the total weight of the instrument and make the bearings of the sliding tube smaller Finally a better balance for calibration of the force control mechanism is to be desired.

Table IV *Fibro-elasticity of the test materials expressed by the value of F*

Test material	F
Foam-rubber material A	0.34
Foam-rubber material B	0.20
Rubber balloon filled with 110 ml of water	0.27
Rubber balloon filled with 180 ml of water	0.19
Uterine cervix in vivo	0.25
Uterine cervix in vitro	0.16

A simplified version of this instrument might be used for measurements of ocular tension. When the instrument is used in a vertical position a simple weight may be used as the force instead of the more complicated force control mechanism. In that case the indicator mechanism could be used without friction between the indicator wings, since it is then possible to read the value of α directly during measurements in such cases.

The instrument has been used for experimental work since 1970 Different materials as well as the cervix of human uteri both in vivo and in

INTRAARTERIAL BLOOD PRESSURE AND PERIPHERAL VASCULAR RESISTANCE IN THE LEG AND ARM DURING THE FIRST TRIMESTER OF PRIMIPREGNANCY

Bo Sandström and Gustav v. Schmalensee

From the Department of Obstetrics and Gynaecology (Head: Professor Per Lundström) and the Department of Clinical Physiology (Head: Professor Håkan Linderholm), University of Umeå, Sweden

Abstract. The intraarterial blood pressure was measured at rest and during reactive hyperaemia of the lower legs in seven primigravidae during the first trimester and in six subjects 2 weeks after early legal abortion. Blood pressure measurements were taken in the right brachial artery and in both femoral arteries. Under conditions of rest the mean blood pressure in the femoral artery in relation to the mean pressure in the brachial artery was lower during early pregnancy than 2 weeks after abortion. The peripheral vascular resistance per 100 ml tissue was calculated on the basis of earlier blood flow investigations and the mean arterial blood pressures reported here. At rest it was found to be higher in the arm than in the leg. During reactive hyperaemia in the lower legs the peripheral vascular resistance was found to be almost the same during early pregnancy and 2 weeks after early abortion.

During the first trimester of pregnancy the resting blood flow is essentially unchanged in the forearm (11) and in the lower leg (7) compared with the blood flow in the nonpregnant state. Blood flow during reactive hyperaemia in the lower legs is significantly lower during early pregnancy than 2 weeks after early abortion. In order to analyse the peripheral haemodynamics, it is necessary to know the blood pressure levels under corresponding experimental conditions.

Summarizing a number of indirect blood pressure examinations of the arm, Hytten & Leitch (3), state that the systolic pressure remains essentially unaltered, while the diastolic pressure falls during the first part of pregnancy. Calculations based on direct measurements of the blood pressure in the brachial artery and cardiac output measured by the direct Fick method, show that the vascular resistance of the systemic circulation is lower during the first half of pregnancy than in the nonpregnant state (2, 8).

Using indirect measurements of the brachial blood pressure in connection with blood flow determinations, Spetz (11) was not able to demonstrate any change in the peripheral resistance of the arm in women during early pregnancy compared with nonpregnant women.

No blood pressure measurements in the leg during early pregnancy are available. For this reason the blood pressure in the femoral and brachial arteries was determined during early pregnancy and after early abortion both at rest and during reactive hyperaemia in the lower legs. The investigation was done to make possible calculation of the peripheral vascular resistance in leg and arm.

MATERIAL

Twenty primigravidae in early pregnancy or just after legal abortion participated in the investigation. The patients were told how the trial was to be conducted and gave their full consent.

In five of the examinations performed during early pregnancy and two following abortion, recordings were not obtained from all three measuring sites (the right brachial artery, the right femoral artery and the left femoral artery). For this reason no arterial blood pressure readings from these subjects have been included in the calculations. Thus, the arterial blood pressures of seven pregnant subjects and those of six subjects after early abortion were evaluated. The abortion was effected by means of vacuum aspiration, the procedure of which has previously been described (4). There were no complications in connection with the abortions. Some characteristics of the two groups are indicated in Table 1.

19 – 73794

BOOKS RECEIVED

Atlas of Colposcopy by Per Kolstad and Adolf Stafl. Universitetsforlaget, Oslo, Norway 1972, 146 pp. 186 illustr. Price 160,— Norw.kr

An excellent book, the best available at present, which ought to be included in the library of every department of obstetrics and gynaecology where colposcopy is performed, and directed biopsies made.

Perinatale Atmung by H. Bartels, K. Riegel, J. Wenner and H. Wolf. Springer Verlag, Berlin 1972, 101 pp. Price 22.— DM US $7.00

The authors represent the specialities obstetrics, paediatrics and physiology and the teamwork they have done is a small but complete book suitable for the teaching in perinatology

Table II. Mean arterial blood pressure during early pregnancy and after abortion measured in the right brachial artery (RBA) and the right and left femoral arteries (RFA, LFA) at rest and during reactive hyperaemia in the lower legs

Mean ± standard error of the mean

	Pregnancy (pr) (n=7)	After abortion (apr) (n 6)	Difference (pr apr)
First measurement (rest with distal leg occlusion)			
Heart rate, beats/min	77 ±3.0	81 ±4.9	−4.0
RBA mean pressure, mm Hg	83.5±1.8	88.5±2.7	−5.2
RFA mean pressure, mm Hg	76.7±1.7	86.8±2.1	−10.1
LFA mean pressure, mm Hg	78.0±2.3	88.0±2.4	−10.0
Mean RFA and LFA (FA)	77.4±2.8	87.4±2.2	−10.0
Difference RBA–FA	5.9*±1.8	1.0±0.7	+ 4.9
Second measurement (reactive hyperaemia in the lower legs)			
Heart rate, beats/min	85 ±3.4	91 ±3.3	−6.0
RBA mean pressure, mm Hg	84.7±2.3	88.8±1.5	−4.1
RFA mean pressure, mm Hg	69.6±2.5	69.2±3.6	0.4
LFA mean pressure, mm Hg	67.7±4.9	70.7±1.7	−3.0
Mean RFA and LFA (FA)	68.6±3.5	69.9±2.4	−1.3
Difference RBA FA	16.2* ±3.9	18.9 ±3.1	−2.7
Third measurement (rest with distal arm occlusion)			
Heart rate, beats/min	76 ±2.6	80 ±4.5	4.0
RBA mean pressure, mm Hg	84.0±2.2	86.5±2.4	−2.5
RFA mean pressure, mm Hg	77.0±2.5	83.7±2.7	−6.7
LFA mean pressure, mm Hg	77.3±2.1	83.8±2.9	−6.5
Mean RFA and LFA (FA)	77.1±2.2	83.8±2.7	−6.7
Difference RBA–FA	6.9* 2.7	2.0±1.3	4.1

(0.01 P 0.05), (0.001 P 0.01).

At the first recording session during early pregnancy the systolic pressure, the diastolic pressure and the mean blood pressure in the brachial artery showed no statistically significant difference from the corresponding blood pressures after early abortion.

Under conditions of rest and during reactive hyperaemia in the lower legs there was no difference in the mean arterial pressures of the right and left femoral arteries—either during early pregnancy or after early abortion (Table II).

At rest (first measurement) the mean blood pressure during pregnancy was lower in the femoral than in the brachial artery. After abortion no corresponding difference was found (Fig.). The mean arterial blood pressure in the femoral artery was thus lower during pregnancy than after abortion.

During reactive hyperaemia in the lower legs the mean blood pressure in the femoral artery relative to the simultaneously recorded pressure in the brachial artery was the same during early pregnancy and following early abortion. In both groups the pressure was lower in the femoral than in the brachial artery.

The mean heart rate was almost the same for both groups of women under all recording conditions.

DISCUSSION

The case material studied consisted only of primigravidae in order to facilitate comparisons with studies of the blood flow in the lower legs published earlier (7). The small difference in the average ages of the groups was considered to have no significant effect on the blood pressure (10). The physiological changes that the women in the control group underwent during the short period of pregnancy (10–12 weeks) should have already disappeared 2 weeks *after* abortion, for which reason the time following this period may be con-

Table I *Some characteristics of the two groups investigated*

Mean ± S D Ranges in brackets

	Pregnancy	After abortion
Age, years	17 1±2.7 (15–21)	21.2±3.5 (17 26)
Menarche, years	12.9±0.7 (12 14)	12.2±1 7 (10–15)
Duration of pregnancy weeks	11.0±1.2 (10–13)	11 0±1 1 (10–12)
Time of examination, days after abortion		13 7±2.9 (11–19)

METHOD

Direct measurements of the arterial blood pressure were preferred in order to avoid errors involved in the indirect method. The arterial blood pressure determinations were designed to render the results comparable to the plethysmographically recorded flow values reported earlier. Five mg of diazepam was administered orally 2 hours before the examination (7). During the measurements of arterial blood pressure the plethysmographs were not applied.

Following local anaesthesia by means of 0.5% Lidocain, the right brachial artery was punctured at the level of the intercondylar line, and a teflon catheter (PE 160) was inserted 4 cm percutaneously (9) in the proximal direction. Teflon catheters were inserted distally in the femoral arteries at the level of the inguinal ligament with the same technique. Employing a steel-wire guide, the tip of the catheter was localized by fluoroscopic control and positioned at knee-joint level.

The blood pressure was recorded on a Mingograf 81 (Elema-Schönander) with a pressure transducer and amplifier. The recording system showed good linearity over the 0 200 mmHg range. The pressures were referred to a zero level at midthoracic height. A common hydrostatic reference level for all the transducers was used.

The heart rate was simultaneously determined from ECG recordings.

After insertion of the catheters the subjects rested for 30 min in order to regain as basal a condition as possible. The arterial pressures and the heart rate were then recorded under conditions of rest with distal arterial occlusion cuffs on both legs (first measurement). After this, reactive hyperaemia was induced simultaneously in the lower legs by means of arterial occlusion cuffs applied to the thighs for 5 min with ischaemic exercise on a foot ergometer (1) during the first 4 min and the same measurements were performed (second measurement). The arterial pressure and heart rate were then recorded at rest with an arterial occlusion cuff applied distally on the right arm (third measurement). The mean blood pressure during reactive hyperaemia was determined 30 sec after releasing the occlusion cuff (7).

RESULTS

Recordings of blood pressure curves and ECG in one case are shown in Fig. 1

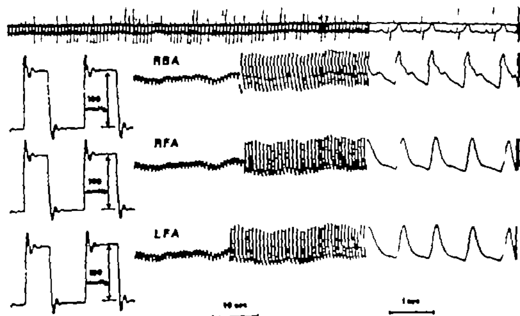


Fig 1 Blood pressure curves for the right brachial artery (*RBA*) and the right and left femoral arteries (*RFA LFA*) Simultaneous ECG recording at the top of the picture

blood pressure in the brachial artery was almost as high during early pregnancy as after early abortion. This agrees with earlier observations (see 5).

In evaluating the peripheral vascular resistance blood flows and pressures (7, 11) were measured under slightly different conditions. The room temperature was the same (22 C), but in the blood flow measurements the forearm and lower legs were surrounded by water at temperatures of 36 and 37°C respectively in the plethysmographs. The difference of one degree in water temperature was considered insignificant. That the blood flow measurements in the forearm (11) ere performed in a case series of both primigravidae and multigravidae was considered not to influence the result (7).

The peripheral vascular resistance was expressed as the quotient of the mean arterial blood pressure in mmHg (as determined in this investigation) and the blood flow in ml/min × 100 ml tissue (as determined in the forearm by Spetz and in the lower leg by Sandström) (Table III). In early pregnancy at rest the vascular resistance is higher in the arm than in the leg and in non-pregnant women t is almost the same. From these findings one might draw the conclusion that the blood essels of the arm during early pregnancy are under the influence of a higher vasoconstrictor tonus than the blood vessels of the leg.

The vascular resistance in the lower legs t rest is the same during pregnancy and after abortion. The mean arterial blood pressure is, however, slightly lower in the femoral artery during pregnancy. This has not been pointed out earlier and could be due to a changed distribution of blood flow in the lower part of the body (kidneys, uterus) during early pregnancy resulting in lower arterial pressure in the legs.

A decrease in blood pressure during reactive hyperaemia is already known to occur (see 1). A small decrease in mean blood pressure together with a small increase (however insignificant) in vascular resistance may explain the decrease in hyperaemic blood flow in the lower legs during early pregnancy reported earlier (7).

REFERENCES

1. Åstrand, P.-O. & Rodahl, Kaare: Textbook of Work Physiology. McGraw-Hill, New York, 1970.
2. Bader, R. A., Bader, M. E., Rose, D. J. & Braunwald, E.: J Clin Invest 34, 1524, 1955.
3. Bevegård, S., Cloetens, E., Jonsson, B. & Karlöf, L.: Scand J Clin Lab Invest 18, 473, 1966.
4. Holmberg, N. G. & Sandström, B.: Lecture at Congress of the Swedish Med. Assoc., Stockholm, 1970. Acta Obstet Gynec Scand 51, 143, 1972.
5. Hytten, F. E. & Leitch, Isabella: The Physiology of Human Pregnancy. Blackwell Scientific Publications, Oxford, 1964.
6. Öbrink, K. J.: Acta Physiol Scand, Suppl. 51, 1948.
7. Sandström, B.: Acta Obstet Gynec Scand 52, 199, 1973.
8. Schwarcz, S. B. de, Aramendia, P. & Taquini, A. C.: Medicina (B. Aires) 24, 113, 1964.
9. Seldinger, I. L.: Acta Radiol (Stockh.) 39: 367, 1953.
10. Smirk, F. H.: High arterial pressure. Blackwell Scientific Publications, Oxford, 1957.
11. Spetz, S.: Acta Obstet Gynec Scand 43, 309, 1964.
12. Thulesius, O.: Scand J Clin Lab Invest 15, 550, 1963.

Submitted for publication Febr. 10, 1972

Bo Sandström
Department of Obstetrics and Gynaecology
University of Umeå
S-901 85 Umeå
Sweden

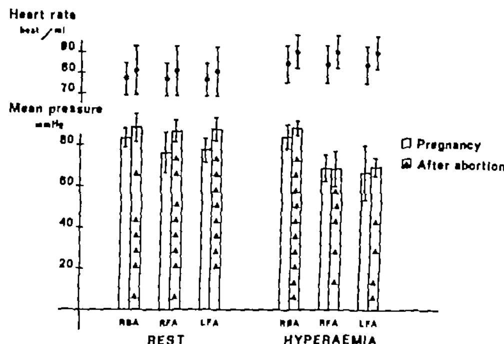


Fig Mean arterial blood pressures (columns) and heart rates (dots) during pregnancy and after abortion—at rest and during reactive hyperaemia in the lower legs. *RBA* Right brachial artery *RFA* right femoral artery *LFA* left femoral artery Vertical lines represent standard deviation (S.D)

sidered as representing nonpregnant conditions (7)

The catheterization procedure may affect the results since varying degrees of tension may be induced in the patients. In order to avoid this effect 10–15 min of rest have been recommended following insertion of the catheter before recording the haemodynamic measurements (3) Since the patients participating in the present investigation were allowed to rest for a period of 30 min after insertion of the last catheter before any measurements were recorded this type of error is probably small.

The systolic and diastolic blood pressures in the brachial artery during early pregnancy did not differ significantly from the corresponding values following early abortion. This does not entirely agree with the results of earlier investigations (see 5) in which a decrease in the diastolic pressure was found during early pregnancy The mean

Table III *Ratios between peripheral vascular resistance in leg and arm during pregnancy (pr) and after early abortion or in the nonpregnant state (apr)*

The blood flow values and the standard error of the mean (in brackets) have been taken from earlier publications (7 11) In some cases, when not reported, the standard deviation has been assumed. With the assumption that the standard deviation of single blood flow values in the forearm is of the same magnitude as for single determination in the lower leg and with the number of observations ≥6 the standard error of the mean for the quotients has been estimated approximately (see 6)

Pregnancy (pr) (resting conditions)	After abortion or nonpregnancy (apr) (resting conditions)
Arm 84 0 (±2.2)/2.1 (±0.2) / Leg 77 4 (±2.8)/2.9 (±0.2) = 1.50 (±0.19)	86.5 (± 4)/2 8 (±0 1) / 87 4 (±2.2)/3 2 (±0 3) = 1 13 (±0 17)
Resting conditions	Reactive hyperaemia
Leg$_{pr}$ 77 4 (±2.8)/2.9 (±0.2) / Leg$_{apr}$ 87 4 (±2.2)/3.2 (±0.3) = 0 98 (±0.12)	68 6 (±3 5)/30 8 (±1 5) / 69 9 (±2.4)/33 5 (±1 8) = 1 07 (±0.10)

$P < 0.05$ (P is the probability that chance has caused the deviation of the quotient from 1).

Acta Obstet Gynec Scand 52. 295–298 1973

INTERRELATION OF DIFFERENT PARAMETERS AT CERVICAL MUCUS PENETRATION OF SPERMATOZOA

Magnar Ulstein and Bo Fjällbrant

From the Department of Obstetrics and Gynecology, Östra sjukhuset (Head, Professor Per Bergman), Göteborg, Sweden

Abstract. Linear penetration, number of penetrating spermatozoa and their motility were studied in 400 semen samples using capillary tube method and with cervical mucus as test medium. A close correlation was found between these parameters. All three showed high correlation with proposed "penetration score". Of the semen properties routinely tested sperm motility showed the highest correlation with the penetration parameters.

Spermatozoa should be able to penetrate the cervical mucus in order to reach and fertilize the ovum. Various possibilities influence the ability of spermatozoa to leave the seminal plasma and penetrate the cervical mucus. The main factors influencing sperm penetration are the seminal properties and the quality of the cervical mucus. Cyclic alterations of the cervical mucus provide varying receptability for the spermatozoa. The best conditions for sperm penetration are found at the time of ovulation (1, 10).

The penetration ability of spermatozoa can be studied in vitro by means of slide tests (8) or capillary tube tests (3, 4, 5). For comparison of the results standardized method is required.

With the capillary tube method several parameters of penetration can be investigated, the linear penetration, the number of spermatozoa which penetrate, the motility degree of the penetrating spermatozoa, and the duration of sperm motility. It is not fully known which of these parameters is of greatest importance for fertility. Some authors (3, 7) have stated that the number of spermatozoa is of greater importance than the linear progression of the spermatozoa. It is not sufficient that only few spermatozoa progress rapidly and the others are left behind. These statements seem reasonable. The uterine cavity is large and a large number of spermatozoa should be needed in order to ensure passage of a sufficient number of spermatozoa to the fallopian tubes. The enzymatic activity of many spermatozoa may be needed for penetration of the zona pellucida of the ovum (9).

The aim of the present work was to investigate the correlation between different parameters of sperm penetration through cervical mucus, as measured by a capillary tube method. A "penetration score" was derived to make the calculations easier and more accurate, and the interrelations between this score, the single parameters and the results of routine semen analysis were studied.

MATERIAL AND METHODS

The material consisted of 400 semen samples tested in our laboratory in connection with infertility investigations.

The semen analyses were performed according to the routine of the laboratory (12). Plastic condoms designed for this purpose were used for collecting the semen samples, which were brought to the laboratory within 2 hours after ejaculation. The semen analyses were started immediately on arrival. The volume of the ejaculate, density of spermatozoa, percentage of motile spermatozoa, motility degree, percentage of abnormal spermatozoa, percentage of living spermatozoa, concentrations of fructose and acid phosphatase in the seminal plasma were determined.

The penetration tests were performed by the method of Kremer (6), with some modifications (11). Capillary tubes with internal diameter of 0.6 mm and length of 41 mm were used. Cervical mucus of ovulatory character carefully procured and fulfilling certain criteria (12) was used as test medium. The cervical mucus was drawn into the capillary tubes, and if not used immediately stored at 4°C. When performing the tests incubation at +37°C for 1 hour was used. When reading the tests the linear

Table III. *Coefficients of correlation between parameters of sperm penetration, including penetration score*

	Linear penetration	No. of penetrating spermatozoa	Motility degree of penetrating spermatozoa	Penetration score
Linear penetration	1.00	0.76	0.73	0.88
Number of penetrating spermatozoa		1.00	0.81	0.93
Motility degree of the penetrating spermatozoa			1.00	0.91
Penetration score				1.00

spermatozoa. This was obvious with samples of good quality. Only with samples of poor quality did a single or a few spermatozoa penetrate much further than the others; these samples had a penetration extent lower than that taken as a sign of fertility (5, 12).

Botella-Llusia (3) has stated that for fertilization it is of no importance that the spermatozoa move rapidly but that a sufficiently large number of them should be able to progress to the higher parts of the female genital tract. From the present investigation it appears that the parameters of penetration are so closely correlated with each other that such a statement seems irrelevant. Noyes (9) states that there seems to be an equilibrium between the penetration of spermatozoa to different parts of the female genital tract, and this can explain the importance of the number of spermatozoa which penetrate.

The theories of a filter function or a depot function of the cervical mucus have been matters of discussion (2, 7, 11). The correlation found between penetration and semen properties (12) may indicate that the filtration which occurs in the cervical mucus depends on the semen quality: spermatozoa of poor quality are not able to penetrate.

Kremer (7) found good correspondence between the results of capillary tube tests and those of the Miller–Kurzrok test (8), where the number of penetrating spermatozoa were taken into consideration. These results may also be an expression of the close correlation between linear penetration and number of penetrating spermatozoa.

In previous studies a close correlation was established between the linear penetration and fertility. From the present work it appears that the different parameters of sperm penetration are very closely related to each other. The penetration score expresses all the parameters; thus it should give a safer measure of the penetration than one parameter read alone. Since all the parameters were almost equally related to the penetration score and since the linear penetration can be measured more accurately than the other parameters, it should be preferred for the judging of fertility by routine investigations.

REFERENCES

1. Bergman, P. Spermmigration and cyclic changes in cervical mucus. Fertil Steril 4: 183, 1953.
2. — Spermmigration and its relation to the morphology and motility of spermatozoa. Int J Fertil 1: 45, 1955.
3. Botella Llusia, J. Measurement of linear progression of human spermatozoa as index of male fertility. Int J Fertil 1: 113, 1956.
4. Carlborg, L. Determination of sperm migration rate in small samples of cervical mucus. Acta Endocr 62: 732, 1969.
5. Fjällbrant, B. Interrelation between high levels of sperm antibodies, reduced penetration of cervical mucus by spermatozoa, and sterility in men. Acta Obstet Gynec Scand 47: 102, 1968.
6. Kremer, J. A simple sperm penetration test. Int J Fertil 10: 209, 1965.
7. — The in vitro spermatozoal penetration test in infertility investigations. Dissertation. University of Groningen, The Netherlands, 1968.
8. Miller, E. & Kurzrok, R. Biochemical studies of human semen. III. Factors affecting migration of sperm through the cervix. Amer J Obstet Gynec 24: 19, 1932.
9. Noyes, R. W. Sperm transport. Progress in infertility (ed. S. Behrman and R. Kistner). Little, Brown and Company, Boston, 1968.
10. Odeblad, E. The functional structure of human cervical mucus. Acta Obstet Gynec Scand 47: Suppl. 1: 57, 1968.
11. Perloff, W. & Steinberger, E. In vivo survival of spermatozoa in cervical mucus. Amer J Obstet Gynec 88: 278, 1960.
12. Ulstein, M. Evaluation of the capillary tube sperm penetration method for fertility investigations. Acta Obstet Gynec Scand 51: 287, 1972.

Table I *Classification of the penetration parameters to construct a penetration score*

The score = the sum of points given for all three parameters

	Points		
	0	1	2
Linear penetration (mm/1 hour)	<5	6–19	>20
Number of penetrating spermatozoa (see text)	0	<20	>20
Motility degree of the penetrating spermatozoa	*0*	*1.2*	*3 4*

penetration of the foremost spermatozoa was given in mm. *The number of spermatozoa which could be seen at* 100× magnification in a visual field just above the edge of the semen reservoir about 5 mm from the immersed end of the capillary tube, was given. The motility degree of the penetrating spermatozoa was classified from 0 to 4 where *0 = no motile spermatozoa, 1 = motility in loco with*out forward movement, 2 = slow 3 = rapid, and 4 = very rapid forward movement. Table I shows how the penetration score was arrived at.

Statistical calculations were made by multiple regression analysis in order to determine the correlation between the penetration parameters, the penetration score and the semen properties.

RESULTS

Table II gives the coefficients of correlation between the semen properties and the different parameters of penetration as well as the penetration score Each semen property was almost equally correlated with the parameters and the penetration score. The number of penetrating spermatozoa and the penetration score showed a slightly higher correlation with the semen properties than the other parameters. The motility degree and the percentage of motile spermatozoa were better correlated with all three parameters and with the penetration score than the other semen properties. Table III shows that the penetration parameters were closely and almost equally correlated with each other and with the penetration score

DISCUSSION

In the present investigation a close correlation was found between the parameters of penetration and the proposed penetration score Each of the parameters and the penetration score had about the same correlation with the semen properties. In the literature no comparative investigations are found Kremer (7) made a classification of *penetration where linear penetration number of* spermatozoa which had penetrated a certain distance, motility degree and duration of motility were taken into consideration but he did not *compare these parameters with each other*

The linear penetration tested by the capillary tube method correlates with fertility (12), but the influence of the other parameters of penetration on fertility has not been tested with this technique. Some authors (3 7) have mentioned that only a few spermatozoa of high vitality may penetrate a longer distance while the others remain far behind From this and previous works (12, 13 14) it is evident that this hardly ever occurs. At the reading of the tests the foremost spermatozoa formed a clear boundary containing numerous

Table II *Coefficients of correlation between semen properties and sperm penetration findings*

Semen property	Linear penetration	No. of penetrating spermatozoa	Motility degree of penetrating spermatozoa	Penetration score
Volume	−0.07	0.08	0.08	0.10
Density	0.23	0.31	0.23	0.28
Percentage of living spermatozoa	0.23	*0.25*	*0.19*	0..3
Percentage of motile spermatozoa	0.41	0.49	0.41	0.46
Motility degree	0.42	0.50	0.42	0.50
Percentage of bnormal spermatozoa	−0.08	−0.21	−0.13	0.16
Fructose concentration	0.02	−*0.11*	*0.04*	0.04
Acid phosphatase concentration	−0.03	*0.02*	*0.02*	0.01

Table III. *Coefficients of correlation between parameters of sperm penetration, including penetration score*

	Linear penetration	No. of penetrating spermatozoa	Motility degree of penetrating spermatozoa	Penetration score
Linear penetration	1.00	0.76	0.75	0.88
Number of penetrating spermatozoa		1.00	0.81	0.92
Motility degree of the penetrating spermatozoa			1.00	0.91
Penetration score				1.00

spermatozoa. This was obvious with samples of good quality. Only with samples of poor quality did a single or a few spermatozoa penetrate much further than the others; these samples had a penetration test lower than that taken as a sign of fertility (5, 12).

Botella-Llusia (3) has stated that for fertilization it is of no importance that the spermatozoa move rapidly but that a sufficiently large number of them should be able to progress to the higher parts of the female genital tract. From the present investigation it appears that the parameters of penetration are so closely correlated with each other that such a statement seems irrelevant. Noyes (9) states that there seems to be an equilibrium between the penetration of spermatozoa to different parts of the female genital tract, and this can explain the importance of the number of spermatozoa which penetrate.

The theories of a filter function or a depot function of the cervical mucus have been matters of discussion (2, 7, 11). The correlation found between penetration and semen properties (12) may indicate that the filtration which occurs in the cervical mucus depends on the semen quality: spermatozoa of poor quality are not able to penetrate.

Kremer (7) found good correspondence between the result of capillary tube tests and those of the Miller–Kurzrok test (8), where the number of penetrating spermatozoa were taken into consideration. These results may also be an expression of the close correlation between linear penetration and number of penetrating spermatozoa.

In previous studies a close correlation was established between the linear penetration and fertility. From the present work it appears that the different parameters of sperm penetration are very closely related to each other. The penetration score expresses all the parameters; thus it should give a safer measure of the penetration than one parameter read alone. Since all the parameters were almost equally related to the penetration score and since the linear penetration can be measured more accurately than the other parameters, it should be preferred for the judging of fertility by routine investigations.

REFERENCES

1. Bergman, P. Spermigration and cyclic changes in cervical mucus. Fertil Steril 4: 183, 1953.
2. — Spermigration and its relation to the morphology and motility of spermatozoa. Int J Fertil 1: 45, 1955.
3. Botella-Llusia, J. Measurement of linear progression of human spermatozoon as index of male fertility. Int J Fertil 1: 113, 1956.
4. Carlborg, L. Determination of sperm migration rate in small samples of cervical mucus. Acta Endocr 62: 732, 1969.
5. Fjallbrant, B. Interrelation between high levels of sperm antibodies, reduced penetration of cervical mucus by spermatozoa, and sterility in men. Acta Obstet Gynec Scand 47: 102, 1968.
6. Kremer, J. A simple sperm penetration test. Int J Fertil 10: 209, 1965.
7. — The in vitro spermatozoal penetration test in infertility investigations. Dissertation. University of Groningen, The Netherlands, 1968.
8. Miller, E. & Kurzrok, R. Biochemical studies of human semen. III. Factors affecting migration of sperm through the cervix. Amer J Obstet Gynec 4: 19, 1932.
9. Noyes, R. W. Sperm transport. Progress in infertility (ed. S. Behrman and R. Kistner). Little, Brown and Company, Boston, 1968.
10. Odeblad, E. The functional structure of human cervical mucus. Acta Obstet Gynec Scand 47: Suppl. 1, 57, 1968.
11. Perloff, W. & Steinberger, E. In vivo survival of spermatozoa in cervical mucus. Amer J Obstet Gynec 88: 278, 1964.
12. Ulstein, M. Evaluation of the capillary tube sperm penetration method for fertility investigations. Acta Obstet Gynec Scand 51: 287, 1972.

13 — Sperm penetration of cervical mucus as criterion of male fertility Acta Obstet Gynec Scand 51 335 1972.
14 — Fertility of husbands at homologous insemination. Acta Obstet Gynec Scand 52 5 1973
15 — Fertility of donors at heterologous insemination. Acta Obstet Gynec Scand 52 97 1973
16 — Fertility motility and penetration in cervical mucu of freeze-preserved human spermatozoa. Acta Obste Gynec Scand 52 205 1973

Submitted for publication March 20 197

Magnar Ulstein
Fylkesjukehuset i Alesund
6017 Asestranda
Norway

Acta Obstet Gynec Scand 52 299–302, 1973

HEPATIC AND ADRENOCORTICAL FUNCTION DURING CYCLOPHOSPHAMIDE STOSS THERAPY

Pekka Ylöstalo, Antti Kauppila, Pentti Jouppila, Kalevi Kivinitty and Allan Vehaskari

From the Department of Obstetrics and Gynaecology (Head, Prof P. A. Järvinen), University of Oulu, Oulu, Finland

Abstract. Forty-one patients with ovarian cancer were investigated for the effect of cyclophosphamide stoss therapy on liver function. The patients were tested for serum bilirubin, alkaline phosphatase, aminotransferases (GOT, GPT), serum total proteins, and ten of them also underwent an intravenous galactose tolerance test. Serum total protein was found to decrease significantly during the cyclophosphamide stoss therapy. It was further noted that the number of pathological results for serum total bilirubin, alkaline phosphatase, GOT and GPT increased during the treatment, whereas the number of pathological half lives of galactose tolerance decreased. Fifteen patients underwent 19 intramuscular Synacthen tests before the beginning of cyclophosphamide stoss therapy and one and two weeks after the therapy. The responsiveness of the adrenal cortex two weeks after the therapy was found to be lower and shorter in duration, compared with the results obtained before the therapy. Cyclophosphamide stoss therapy was not found to cause any serious disorders of hepatic or adrenocortical function.

During the last few years, increasingly large single doses have been introduced in intravenous cyclophosphamide treatment. Single doses of 1 000 mg cyclophosphamide (Sendoxan Pharmacia) administered intravenously every second day have been used in the Clinic of Gynaecology, University of Oulu. Administration is discontinued when a total dose of 5 g is reached, or leucocyte counts of less than 2 000/mm^3 are recorded. The cyclophosphamide stoss therapy was given in courses at intervals of 1–6 months. A new course was not started until leucocyte count had exceeded 3 000/mm^3. The purpose of the present work was to elucidate the possible hepato- and adrenotoxic effects of such large doses of cyclophosphamide.

Cytostatic agents have established their position in the treatment of malignant diseases. One of the most common is cyclophosphamide (2H-1,3,2-oxazaphosphorine, 2-bis [2-chloroethyl]) aminotetrahydro-, oxide, hydrate), which can be administered in different doses (6). Cyclophosphamide has an inhibiting effect on the DNA synthesis, for which reason the rapidly increasing tumour cells are sensitive to the effect of this drug. Being a cytotoxic, however, it has several harmful effects on normal tissues. The most powerful side-effect is on the bone marrow, which may lead to leucopenia, depending on the dose of the cytostatic agent used. The other side-effects include loss of hair, nausea, vomiting, hepatic and renal injury, skin rashes, and haemorrhages in the urinary bladder (1, 2, 5, 8, 11).

MATERIAL AND METHODS

Liver function

The series consists of 41 patients (age range 45–73 years) with ovarian cancer confirmed histologically. Before starting the cyclophosphamide stoss therapy the following liver function tests were performed: serum bilirubin, transaminases (GOT, GPT), alkaline phosphatase, thymol, serum total proteins and electrophoretic distribution of proteins. The methods for the tests have been presented earlier (16). The liver function tests were repeated when the cyclophosphamide treatment was discontinued for the first time, and again after the prescribed amount of the drug had been administered. The treatment was given in courses, of which there were 2.3 on average (range 1–6), the average amount of cyclophosphamide administered being 4.9 g (2–6 g) during the first course and 10.1 g (2–23 g) during the whole period of treatment. In addition to this, 10 patients (selected at random) underwent an intravenous galactose tolerance test before and after the first course of cyclophosphamide. The test

Table I *Results of the liver function tests of 41 ovarian cancer patients*

A, Before cyclophosphamide treatment B, after the first course of treatment (average 4 9 g) C, after the whole period of treatment (average 10.1 g)

Liver function test	A (M ± S E.)	B (M ± S E.)	C (M ± S E.)
Serum total bilirubin (mg/100 ml)	0.27 ± 0 05	0 66 ± 0.29	0.66 ± 0.29
GOT (Wroblevski units/ml)	27 4 ± 2.9	32.7 ± 2.8	26.4 ± 1.8
GPT (Wroblevski units/ml)	17 9 ± 2.9	25.2 ± 3.3	19 4 ± 2.4
Alkaline phosphatase (B–L units)	2.08 ± 0 18	2.31 ± 0.21	2.33 ± 0.21
Thymol (Maclagan units)	1.36 ± 0.15	1.03 ± 0 11	1 08 ± 0 14
Serum total protein (g/100 ml)	7.36 ± 0.13	6 87 ± 0.11	6 98 ± 0.13
Electrophoretic distribution of serum proteins (%)			
albumin	48.0 ± 1 4	49 9 ± 1 4	50.2 ± 1 4
α -globulin	5 4 ± 0.3	5 1 ± 0 4	4.8 ± 0.3
α_2-globulin	13 5 ± 0.6	13.5 ± 0.6	12.4 ± 0.7
β-globulin	12.2 ± 0 3	1[illegible]6 ± 0.4	12.2 ± 0 4
γ-globulin	20 9 ± 1 0	19 7 ± 0.8	20.5 ± 0 8

consisted of rapid i travenous infusion of 350 mg f sterile 30% galactose solution per kg of body weight dur i g a period of /$_2$–2 minutes. Finger tip blood samples were obtained once before the test and after the test at 10 min intervals up to 60 min. Galactose content was determined enzymatically (14). The half lives of galactose were calculated from a semilogarithmic table according to the method described by Tengström (13).

Adrenocortical function

Fifteen patients undergoing cyclophosphamide stoss ther apy were tested, four of them twice. Four of the cases investigated were receiving their first course f treat men, five th-ir second course and 10 their 3rd–6th course of treatment. Adrenocortical f nction was tested using synthetic polypeptide$_{1-24}$ 1 24 ACTH (Itomactud) The patient had a light breakfast. Synthetic ACTH (10 μg) was injected intramuscularly at 1 o clock Samples of venous blood were obtained immediately prior to the injection, and later 30 min and 60 mi after the i jection. The cortisol content f the venous blood samples was de termined by the photofluorometric method developed by De Moor et al. (3) and modified by Laurell (7). The ACTH test was performed i each case before the be ginning of treatment, one week fter stopping the treat ment and again two week after stopping the treatment.

RESULTS

Liver function

The results for serum bilirubin aminotransferases (GOT GPT) alkaline phosphatase thymol serum total protein and electrophoretic distribution of proteins are presented in Table I The decrease of total protein was significant ($p < 0.01$) after the first course of cyclophosphamide stoss therapy and almost significant ($p < 0\,05$) after the whole period The doubling of the mean noted in the serum bilirubin values was not statistically significant ($p > 0\,05$). The number of pathological results obtained for the liver func tion tests are presented in Table II It can be seen that the number of pathological results for serum total bilirubin alkaline phosphatase GOT and GPT slightly increased during the treatment, particularly during the first course The electro- phoretic distribution of serum proteins, on the other hand shows hardly any change in the fre quency of pathological results. Table III shows the results of the galactose tolerance tests before and after the first course of cyclophosphamide stoss therapy Values of over 17 min which can

Table II *Number of pathological results in the liver function tests of 41 ovarian cancer patients*

A, Before cyclophosphamide treatment B, after the first course of treatment (erage 4 9 g) C, after the whole pe riod of treatment (average 10.1 g)

	Number of pathological results		
Liver functio test	A	B	C
Serum total bilirubin (over 1.[illegible] mg/100 ml)	1	3	2
GOT (over 40 Wroblevski units/ml)	6	10	4
GPT (over 30 Wroblevski units/ml)	7	9	8
Alkaline phosphatase (over 90 B–L units)	5	7	9
Thymol (over 4 Maclagan units)	1	0	0
Serum total protein			
Increased (over 8 2 g/100 ml)	7	1	2
Decreased (under 6 6 g/100 ml)	9	13	11
Serum albumin			
Increased (over 67)	0	1	1
Decreased (under 53)	25	28	21
Serum α -globulin			
Increased (er 4 4)	26	20	17
Decreased (under 1 9)	0	2	2
Serum α_2-globulin			
Increased (over 10 5)	31	31	23
Decreased (nder 5 0)	0	0	0
Serum β-globulin			
Increased (over 1 5)	17	17	15
Decreased (under 7 1)	0	1	1
Serum γ-globulin			
Increased (over 21 0)	20	19	17
Decreased (nder 10 5)	1	1	1

be considered pathological, were recorded in 4 cases before the treatment and in 2 cases after the first course. The average half-life became slightly but not significantly ($p < 0.40$) shorter.

Adrenocortical function

The results obtained before the treatment (I), one week after the treatment (II) and two weeks after the treatment (III) were analyzed separately. Table IV shows the corresponding absolute values, the increases at 30 min and 60 min as compared with the initial values, and the change between 30 and 60 min. The changes were similar in groups I and II, but the changes recorded at 30 and 60 min in group III were smaller than those in the other two groups. In groups I and II further increase was recorded between 30 and 60 min, but the cortisol level in group III remained almost constant. No statistically significant differences were noted either in the initial values or in the ones obtained at 30 and 60 min. Neither were the differences in the increments statistically significant. The increase from 0 to 60 min in group III, however, was noticeably smaller than that in the group I ($p < 0.1$).

DISCUSSION

It can be concluded on the basis of the results that the hepatotoxicity of cyclophosphamide is so slight that it does not significantly complicate the treatment. Since, however, some serious complications have been reported (1), it is important to

Table III *Intravenous galactose tolerance tests of 10 ovarian cancer patients before (A) and after (B) the first course of cyclophosphamide treatment*

Patients	Half life of galactose (min)	
	A	B
1 S K	1	17
2 I H	12	13
3 A S	27	17
4 H L	10	9
5 A T	22.5	15
6 I T	12.5	14
7 J A	10	7.5
8 F R	1	13.5
9 A T	8	27.5
10 R S	15	9
Mean ± S.E.	13.6 ± 1.8	14.7 ± 1.9

Table IV *Plasma cortisol levels and increments during the HOMACTID test in the different groups (19 tests for 15 patients)*

		Absolute values (µg/100 ml)			Increments (µg/100 ml)		
Time (min)		0	30	60	0–30	0–60	30–60
I	Mean	22.5	41.7	47.4	19.2	24.9	5.7
	S.E.	3.1	5.8	4.7			
II	Mean	20.8	38.1	43.3	17.3	22.5	5.2
	S.E.	3.4	4.4	5.2			
III	Mean	22.7	37.4	37.9	14.7	15.2	0.5
	S.E.	2.1	2.6	3.5			

ascertain the hepatotoxic effect of large doses of cyclophosphamide in patients who already have disorders in their liver function. It has been considered advisable, at any rate, to avoid cyclophosphamide treatment in cases of acute hepatic parenchymal diseases (4). The slight increase of pathological aminotransferase values noted in the results agrees well with the earlier studies (5), which further report the aminotransferase findings to be accompanied by a temporary decrease of the cholinesterase values. The significant decrease of total protein during the treatment is probably an indication of the direct effect of the active metabolites of cyclophosphamide on the cytoplasmic factors of hepatic protein synthesis (10). Increased half-lives of galactose tolerance have earlier been recorded in hepatitis and liver cirrhosis (13). In the present study 4 patients out of the 10 investigated were found to have pathological half-lives of galactose before the cyclophosphamide treatment. After the first spell of treatment pathological values were noted in only 2 patients. It seems, therefore, that cyclophosphamide treatment has no great effect on the hepatic redox potentials measured by the galactose tolerance test.

The Homactid test has turned out to be sufficiently sensitive for diagnosing adrenocortical dysfunction (12, 15). It has been shown that a 10 µg dose of synthetic ACTH can be administered either intramuscularly or intravenously in the test. Tests after 30 and 60 min have proved sufficiently sensitive (9, 12). It was noted in the 60-min test, in the present work, that about two weeks after stopping cyclophosphamide treatment, when the depressant effect of cyclophosphamide

Table I *Results of the liver function tests of 41 ovarian cancer patients*

A, Before cyclophosphamide treatment B, after the first course of treatment (average 4 9 g) C, after the whole period of treatment (average 10.1 g)

Li er function test	A (M±S.E.)	B (M±S E.)	C (M±S.E.)
Serum total bilirubin (mg/100 ml)	0.27±0.05	0.66±0.29	0 66±0.29
GOT (Wroblevski units/ml)	27 4±2.9	32.7±2.8	76.4±1 8
GPT (Wroblevski units/ml)	17.9±2.9	25.2±3.3	19 4±2.4
Alkaline phosphatase (B–L units)	2.08±0.18	2.31±0.21	2.33±0.21
Thymol (Maclagan units)	*1.36±0.15*	*1.03±0.11*	*1.09±0.14*
Serum total protein (g/100 ml)	7.36±0.13	6.87±0.11	6.98±0.13
Electrophoretic distribution of serum proteins (%)			
albumin	48.0±1 4	49.9±1 4	50.2±1 4
α -globulin	5 4±0.3	5 1±0 4	4 8±0.3
α_2-globulin	13.3±0.6	13.5±0.6	12.4±0.7
β-globulin	*12.2±0.3*	12.6±0 4	12.2±0.4
γ-globulin	20.9±1.0	19 7±0.8	20.5±0 8

consisted of rapid intravenous infusion 1 350 mg of sterile 30% galactose solution per kg of body weight during a period of 1/2-2 minutes. Finger tip blood samples were obtained once before the test, nd after the test at 10 min intervals up t 60 min. G lactose content was determined enzymatically (14). The half lives of galactose were calculated from a semilogarithmic table according t the method described by Tengström (13).

Adrenocortical function

Fifteen patients undergoing cyclophosphamide stoss therapy were tested, four of them twice Four of the cases investigated wer *receiving their first course of tre t* men, f th ir second course and 10 their 3rd–6th course of treatment. Adrenocortical function was tested using synthetic polypeptidea 1–24 ACTH (Homactid) *The patient had a light breakfast. Synthetic ACTH (10 μg)* was injected intramuscularly at 12 clock. Samples of venous blood were obtained immediately prior t the injection, and later 30 min nd 60 min after the injection. *The cortisol content of the venous blood samples was determined* by the photofluorometric method dev loped by De Moor et al. (3) and modified by Laurell (7) The ACTH test was performed in each case before the beginning of treatment, one week *after stopping the treatment* and again two weeks after stopping the treatment.

RESULTS

Liver function

The results for serum bilirubin aminotransferases (GOT GPT) *alkaline phosphatase*, thymol serum total protein, and electrophoretic distribution of proteins are presented in Table I The decrease of total protein was significant ($p<0.01$) after the *first* course of *cyclophosphamide* stoss therapy and almost significant ($p<0.05$) *after the whole period.* The doubling of the mean noted in the serum bilirubin values was not statistically significant ($p>0.05$). The number of *pathological results obtained for the liver function* tests are presented in Table II It can be seen that the number of pathological results for serum total bilirubin alkaline phosphatase GOT and GPT slightly increased during the treatment, particularly during the *first* course The electrophoretic distribution of serum proteins, on the other hand shows hardly any change in the frequency of pathological results. Table III shows *the results of the galactose tolerance tests before* and after the first course of cyclophosphamide stoss therapy Values of over 17 min which can

Table II *Number of pathological results in the liver function tests of 41 ovarian cancer patients*

A, Before cyclophosphamide treatment B, after the first course of treatment (a erage 4.9 g) C, after the hole period of treatment (verage 10.1 g)

Liver function test	Number of pathological results		
	A	B	[illegible]
Serum total bilirubin (over 1.- mg/100 ml)	1	3	
GOT (over 40 Wroblevski units/ml)	6	10	
GPT (over 30 Wroblevski units/ml)	7	9	
Alkaline phosphatase (over 2 90 B–L units)	5	7	
Thymol (over 4 Maclagan units)	1	0	
Serum total protein			
Increased (over 8.2 g/100 ml)	7	1	
Decreased (under 6 6 g/100 ml)	9	13	1[illegible]
Serum albumin			
Increased (over 67)	0	1	
Decreased (under 53)	[illegible]5	28	2[illegible]
Serum α -globulin			
Increased (o er 4 4)	[illegible]6	[illegible]0	1[illegible]
Decreased (under 1 9)	0	2	[illegible]
Serum α_2-globulin			
Increased (over 10.5)	33	31	[illegible]
Decreased (under 5 0)	0	0	[illegible]
Serum β-globulin			
Increased (over 1 5)	17	17	1[illegible]
Decreased (under 7 1)	0	1	1
Serum γ-globulin			
Increased (over 21 0)	20	19	17
Decreased (under 10 3)	1	1	1

be considered pathological, were recorded in 4 cases before the treatment and in 2 cases after the first course. The average half-life became slightly but not significantly ($p < 0.40$) shorter.

Adrenocortical function

The results obtained before the treatment (I), one week after the treatment (II) and two weeks after the treatment (III) were analyzed separately. Table IV shows the corresponding absolute values, the increases at 30 min and 60 min as compared with the initial values, and the change between 30 and 60 min. The changes were similar in groups I and II but the changes recorded at 30 and 60 min in group III were smaller than those in the other two groups. In groups I and II a further increase was recorded between 30 and 60 min, but the cortisol level in group III remained almost constant. No statistically significant differences were noted either in the initial values or in the ones obtained at 30 and 60 min. Neither were the differences in the increments statistically significant. The increase from 0 to 60 min in group III, however, was noticeably smaller than that in the group I ($p < 0.1$).

DISCUSSION

It can be concluded on the basis of the results that the hepatotoxicity of cyclophosphamide is so slight that it does not significantly complicate the treatment. Since, however, some serious complications have been reported (1), it is important to ascertain the hepatotoxic effect of large doses of cyclophosphamide in patients who already have disorders in their liver function. It has been considered advisable, at any rate, to avoid cyclophosphamide treatment in cases of acute hepatic parenchymal diseases (4). The slight increase of pathological aminotransferase values noted in the results agrees well with the earlier studies (5), which further report the aminotransferase findings to be accompanied by a temporary decrease of the cholinesterase values. The significant decrease of total protein during the treatment is probably an indication of the direct effect of the active metabolites of cyclophosphamide on the cytoplasmic factors of hepatic protein synthesis (10). Increased half-lives of galactose tolerance have earlier been recorded in hepatitis and liver cirrhosis (13). In the present study 4 patients out of the 10 investigated were found to have pathological half-lives of galactose before the cyclophosphamide treatment. After the first spell of treatment pathological values were noted in only 2 patients. It seems, therefore, that cyclophosphamide treatment has no great effect on the hepatic redox potentials measured by the galactose tolerance test.

The Homactid test has turned out to be sufficiently sensitive for diagnosing adrenocortical dysfunction (12, 15). It has been shown that a 10 µg dose of synthetic ACTH can be administered either intramuscularly or intravenously in the test. Tests after 30 and 60 min have proved sufficiently sensitive (9, 12). It was noted in the 60-min test, in the present work, that about two weeks after stopping cyclophosphamide treatment, when the depressant effect of cyclophosphamide

Table III *Intravenous galactose tolerance tests of 10 ovarian cancer patients before (A) and after (B) the first course of cyclophosphamide treatment*

Patients	Half life of galactose (min)	
	A	B
1 [illegible]	19	17
2 [illegible]	12	13
3 [illegible]	27	17
[illegible]	18	9
5 [illegible]	22.5	19
6 [illegible]	12.5	14
[illegible]	10	7.5
[illegible]	10	13.5
[illegible]	18	27.5
10 [illegible]	11	9
Mean ± S.E.	15.6 ± 1.8	14.7 ± 1.8

Table IV *Plasma cortisol levels and increments during the HOMACTID test in the different groups (19 tests for 15 patients)*

	Absolute values (µg/100 ml)			Increments (µg/100 ml)		
Time (min)	0	30	60	0–30	0–60	30–60
I Mean	22.5	41.7	47.4	19.2	24.9	5.7
S.E.	3.1	5.8	6.7			
II Mean	20.8	38.1	43.3	17.3	22.5	5.2
S.E.	3.4	4.4	5.2			
III Mean	22.7	37.4	37.9	14.7	15.2	0.5
S.E.	2.1	2.6	3.5			

on the bone marrow is still strong according to peripheral leucocyte count, the responsiveness of the adrenal cortex was depressed and less well sustained if compared with the result obtained before the treatment. This effect is possibly due either to the direct adrenal effect of cyclophosphamide or secondarily to the fact that cyclophosphamide stoss therapy impairs the general condition of the patient. The results seem to suggest that if there are stress situations occurring during or after the cyclophosphamide treatment, administration of corticosteroids may be useful.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Sigrid Jusélius Foundation. We wish also to thank Ferring AB for the gift of Homactid.

REFERENCES

1 Aubrey D A., Massive hepatic necrosis after cyclophosphamide Br Med J 3 588 1970.
2 Dadoni, L. G. & Seoane N R., Toxicidad y efectos secundarios de la ciclofosfamida. Rev Clin Esp 109 11 1968.
3 De Moor B., Steeno, O Raskin, M & Hendrikx, A., Fluorometric determination of free plasma 11 Hydroxycorticosteroids in man. Acta Endocrin (Kbh) 33 297 1960.
4 Hegewald, G Barenwald, G Grisk A. & Möritz, K.-U Elektronenmikroskopische Befunde bei Leberschaden durch Arzneimittel (Choleretika, Zyklophosphamid, Phenylindadion) Z Ges Inn Med 23 821 1968
5 Kayser P Weber H. & Stamm, O Die prognostische und therapeutische Beurteilung des Ovarialcarcinoms anhand des Enzymstatus. Gynaecologia 165 79 1967
6. Kjellgren, O Gynekologisk cancer Klinik och terapi, p. 187 Almqvist & Wiksell, Stockholm, 1967
7 Laurell, C. E., Personal communication, 1970.
8. Lledberg, E. F Rauding, A. & Langeland, P Cyclophosphamide hemorrhagic cystitis. Scand J Urol Nephrol 4 183, 1970.
9 Luton, J P Laudat, P & Bricaire, H., L'emploi de béta 1 24 corticotrophine retard dans d'exploration functionelle du cortex surrénal. Ann Endocrin (Paris) 30: 456, 1969
10. Schnitger F & Uehleke H., Der Einfluss von Dimethylnitrosamin, Tetrachlorkohlenstoff, Buttergelb und Cyclophosphamid auf den Aminosäureneinbau in Fraktionen von Leberhomogenaten nach metabolischer Aktivierung in vitro. Arch Toxik 23 169 1969
11 Starinsky V V Trakhtenberg, A. K. & Batinov L N Radioisotope investigation of the liver function in the cyclophosphan treatment of lung cancer. Med Radiol (Mosk) 15 32, 1970.
12. Still, L., Rennili, M. & Järvinen, P Adrenocorticotrophintest applied on women taking oral contraceptives. Acta Endocrin (Kbh) 67 Suppl. 155 167 1971
13 Tengström, B An intravenous galactose tolerance test with an enzymatic determination of galactose A comparison with other diagnostic aids in hepatobiliary diseases. Scand J Clin Lab Invest 18 Suppl. 92: 132, 1966.
14 Verdier C. H. & de Hjelm, M A galactose oxidase method for the determination of galactose in blood plasma. Clin Chim Acta 7 742, 1962.
15 Woodings, D. F & Young, P A rapid test of adrenocortical function using Homactid (synthetic $^{1-18}_{\beta}$ ACTH). Acta Endocrin 67 470, 1971
16. Ylöstalo, P Liver function in hepatosis of pregnancy and pre-eclampsia with special reference to modified bromsulphthalein tests. Acta Obstet Gynec Scand 49 Suppl. 4 1970.

Submitted for publication June 16 1972

Pekka Ylöstalo
Department of Obstetrics and Gynaecology
University of Oulu
Oulu
Finland

Acta Obstet Gynec Scand 52, 303–307 1973

THE INDUCTION AND STIMULATION OF LABOUR WITH BUCCAL DESAMINOOXYTOCIN AND OXYTOCIN TABLETS

Erik Fangel Poulsen

From the Department of Gynecology and Obstetrics (Heads: Georg Zachariassen, Professor M.D. and Finn Lund ..., M.D.), Øresunds Hospital, Copenhagen, Denmark

Abstract 241 pregnant women were treated with buccal oxytocin tablets (129) and buccal desaminooxytocin tablets (112) respectively with a view to the induction of labour (135) and the stimulation of labour (106). Delivery was achieved within 48 hours after 190 of the treatments (79%). It was found that the dilatation time was significantly shorter and the dosage of biologically active compound required was less, for women treated with desaminooxytocin. Otherwise there was no significant difference between the results of treatment, and the frequency of side-effects and complications was deemed to be of the same magnitude in the two treatment groups.

Extracts from the posterior lobe of the hypophysis have been used in obstetrics as oxytocic agents since 1909. These have often been employed both uncritically and in too large doses with disastrous and dangerous complications, such as fetal death and uterine rupture as the result.

The uterine stimulating factor from the posterior lobe of the hypophysis—oxytocin—has been known in its pure form for almost 20 years. Synthetic oxytocin has been available for almost as long and has been used in obstetrics both to stimulate and to induce labour. In 1960 a synthetic oxytocin derivative—desaminooxytocin—was discovered; this has the same properties as oxytocin.

Du Vigneaud et al., who were the first to synthesize desaminooxytocin (5) and several others have since carried out comparative studies of synthetic oxytocin and desaminooxytocin. In this connection it can in particular be emphasized that

(1) The human uterus is stimulated approximately 1.5 times more strongly by desaminooxytocin than by oxytocin calculated in biological units and approximately twice as strongly calculated by the weight of the drug used. 1 mg of oxytocin is equivalent to 450 I.U. 1 mg of desaminooxytocin to 710 I.U. (1, 2, 4, 7, 9).

(2) The oxytocic effect is qualitatively identical for both compounds (6, 8).

(3) Clinical studies with desaminooxytocin used for induction and stimulation of labour have given similar results to those previously found with both intravenous infusion and the administration of buccal tablets of oxytocin (8, 9, 10, 11).

(4) The frequency of birth complications is not higher with induction and stimulation of labour using desaminooxytocin than when using oxytocin (2).

MATERIAL AND METHODS

A study has been carried out at the department of gynaecology and obstetrics of the Øresunds Hospital. This included all the pregnant women at term who in the period 1967 – March 1969 were subjected to induction of labour or labour stimulation with the help of buccal tablets of oxytocin (hereafter termed SYT) and buccal desaminooxytocin tablets (hereafter termed ODA), kindly provided by Sandoz A.S. SYT was used in 1967 and ODA in 1968–69.

In the first period a total of 129 patients were thus treated with SYT, while a total of 112 patients were treated with ODA in the second period. The two groups comprised the same percentage of the total number of patients in each of the two periods (13.5% and 13.7% respectively).

It was also found that there was good agreement in the two groups between the ages of the mothers, their weight and parity (Table I).

On the other hand there was some variation with regard to the indication for treatment in the two groups (Table II).

For this reason, amongst others, it is necessary to carry out selection of the induction of labour and stimulation of labour when evaluating the results of the

Table I *The age weight and parity of the mothers in the two treatment groups*

	SYT	ODA
Age of mother	23.0 years ±4.6	23 1 years ±4 4
Weight of mother (at term)	70 7 kilo ±8 4	71 3 kilo ±9.9
Parity		
Nullipara (No of Pat.)	90 (70 %)	78 (70 %)
I–III para (No of Pat.)	39 (30 %)	34 (30 %)

treatment. In the group "prolonged pregnancy" only nulliparae who had not given birth 10 days or more after term are included (calculated according to Neegel's rule).

A large number of patients were also subjected to induction of labour for suspected large fetus" (fetal weight >3 700 g) The reasons for the stimulation of labour were ut rine inertia following rupture of the membranes and "secondary uterine inertia

Buccal tablets of various strength were employed in the two treatment groups.

SYT each tablet contained 100 I U of oxytocin.

ODA. each tablet contained 25 I.U of desaminooxytocin.

E aluated according to the biological activity on the human uterus the SYT tablets were thus more than twice as strong as the ODA tablets (see Introduction para 1)

The treatment scheme which was used, however was the same.

1/2 tablet, wait for effect for 30 min, then

1 tablet, wait for effect for 30 min, then

- tablets wait for effect for 30 min, then

1 tablet, wait for effect for 30 min, then

2 tablets wait for effect f r 30 min and so on, however a maximum of 6 tablets or the remains f tablets in the mouth at one time

A maximum of 19 1/2 tablets per day was given. The treatment was discontinued as soon as regular labour

Table II *Patients in the two treatment groups according to the indication for treatment*

	SYT	ODA
Induction of labour	81 (63 %)	54 (48 ")
Of these prolonged pregnancy	29 (23 %)	24 (21 %)
Other reasons for induction	52 (40 %)	30 (?6 %)
Suspected large fetus	21	14
High-risk pregnancy	9	4
Breech presentation	7	3
Preeclampsia	4	4
Small stature	4	2
Other indications	7	3
Stimulation of labour	48 (37 %)	58 (52 %)
Of these uterine nertia following membrane rupture	37	40
Secondary uterine inertia	11	/ 18

Table III *Retrospective evaluation of the pelvic score in the two treatment groups at the beginning of the treatment*

	SYT		ODA	
Pelvic score	No of pats.	%	No of pats.	
0–6	39	30	61	55
7 9	59	46	36	32
10–12	31	24	15	13

pains occurred. If the attempted treatment was not successful on the first day then it was repeated on the following day An attempt was termed successful, if the patients were delivered within 48 hours after the beginning of the first course of tablets.

Vaginal examination was performed prior to an attempt at treatment. The findings of this examination were noted in the case history They were not used as one of the indications for induction, but entirely for the evaluation of the possible outcome of the treatment.

The examination findings have been transformed retrospectively to "pelvic score according to the method of Bishop (3). The results can be seen in Table III.

Artificial rupture of the membranes was not performed at the beginning of the treatment. Providing spontaneous rupture did not occur then artificial rupture was performed when "there were good regular contractions when the cervix was effaced and there as at least 3 cm of dilatation (T ble IV).

RESULTS AND EVALUATION

Various methods have been employed to determine the efficacy of the treatment

(1) By the number of successful attempts at treatment.

(2) By the number of tablets required, expressed in international units, for successful attempts at treatment.

Table IV *Membrane rupture (artificial or spontaneous) with induction of labour*

	SYT		ODA	
	No of pats.	"	No. of pats.	
Artificial				
Less than 4 hours after begin ing of treatment	8	10	11	20
More than 4 hours fter begin ing of treatment	12	15	8	15
Spontaneous	61	75	35	65
Total	81		54	

Table V. The efficacy of the treatment illustrated by the number of successful attempts (delivery within 48 hours after the beginning of the treatment) distributed according to various indications

	SYT		ODA	
	Delivered	Undelivered	Delivered	Undelivered
Induction of labour				
Prolonged preg.	20	9	16	8
Other induction	35	17	18	12
Stimulation of labour	48	0	53	5
Total number of treatments	103 (80)	26	87 (78 %)	25

(3) By the duration of the first and second stages of labour in successful attempts at treatment.

(1) The results of all the attempts at treatment can be seen in Table V. Evaluated from the number of successful attempts alone there is no significant difference in the results in the two groups ($p > 0.05$).

In this connection, however, it should be noted that the dosage of active compound (100 I.U. oxytocin tablet against 25 I.U. desaminooxytocin/tablet) was reduced to less than half in the attempt at treatment with ODA.

From Table III it can, in addition, be seen that the pelvic score at the beginning of the treatment as, on the whole, lower for the ODA group of patients than for the SYT group of patients. This is of importance because a higher pelvic score

Table VI. Average of the administered dosage expressed in international units and logarithm of the dosage of the successful attempts at treatment

Time to birth after the beginning of the treatment (h)	SYT 84 pat. 0–24	SYT 19 pat. 24–48	ODA 76 pat. 0–24	ODA 11 pat. 24–48
Dosage in I.U.	1 042 604	2 463 499	222 127	709 190
log I.U.	2.91 0.36	3.38 0.12	2.23 0.32	2.83 0.13
log I.U. 2	2.60 0.36	3.08 0.12		

Table VII. The efficacy of the treatment illustrated by the average of the administered dosage expressed in international units, with successful attempts, according to the indication for treatment

Time to delivery after start of treatment (h).	SYT 0–24	SYT 24–48	ODA 0–24	ODA 24–48
Nullipara				
Induction of labour (I.U.)				
Prolonged pregn.	1 419 ±578	2 430 ±746	253 ±162	778 ±200
Other inductions	1 198 ±421	2 400 ±1 002	325 ±177	642 ±232
Stimulation of labour (I.U.)	828 ±614		174 ±102	
Para I–III				
Induction of labour (I.U.)	1 233 ±434	2 863 ±575	331 ±102	778 ±0
Stimulation of labour (I.U.)	843 ±539		205 ±95	

at the beginning of the treatment increases the success of treatment.

A third factor which can considerably influence the results of treatment is the frequency with which artificial rupture of the membranes was carried out in connection with the treatment. It can be seen from Table IV that this factor did not have any decisive influence on the results.

(2) It can be seen from Table VI that the average tablet consumption (expressed in I.U.) in the successful attempts at treatment, and in those delivered within 24 hours after the beginning of the first course of tablets, is lower for ODA groups of patients than for those of SYT group. This difference is statistically significant ($p < 0.001$). It is, however, seen that there is a distorted distribution of the results in the individual groups. A more correct comparative testing of the results is therefore carried out by considering the logarithmic values. In order to further eliminate this difference, which is due to the greater biological activity of ODA ($1.5–2 \times$ SYT) the logarithmic value of half of the SYT dosage is taken but the full value of the ODA dosage. Despite this there is a statistically significant difference ($p < 0.01$) in the results. Or in other words, the dosage was significantly lower with the successful attempts in the ODA group.

A comparison of the results in the two groups

Table VIII *The efficacy of the treatment illustrated by the average of the logarithm of the administered dosage expressed in international units with successful attempts*

The SYT dosage has previously been divided by 2. See text. The results are given according to the indication for treatment

Time to delivery after start of treatment (h)	SYT 0–24	SYT 24–48	ODA 0–24	ODA 24–48
Nullipara				
Induction of labour ("log. I U")				
Prolonged pregn.	2.80 ±0.24	3.07 ±0.12	2.32 ±0.31	2.88 ±0.1
Other indication	2.75 ±0.19	3.06 ±0.16	2.43 ±0.30	2.79 ±0.17
Stimulation of labour ("log I U")	2.48 ±0.37		2.14 ±0.35	
Para I III				
Induction of labour ("log I U")	2.71 ±0.34	3.15 ±0.04	2.50 ±0.14	2.90 ±0
Stimulation of labou ("log I.U.")	2.51 ±0.40		2.25 ±0.25	

where delivery occurred after the 2nd course of tablets (24–48 hours after the beginning of the 1st course of tablets) must be made with reservation as the sum of the tablets consumed during the first course varied aligthly (maximum 19 1/2 tablets) See the directions for the courses of treatment.

A similar evaluation has been made in Tables VII and VIII. The patients are divided into groups according to the indication for treatment. The results are given in average doses expressed in international units. But a test using logarithmic values, has also been carried out here because of the distribution in a similar manner to the one done earlier with the logarithm of half the SYT dosage and the full ODA dosage.

Here also the expression of the ODA dosage is significantly lower ($p < 0.01$) than the expression of the SYT dosage/2. This does not, however apply to the group of inductions of labour in parous patients. It is not possible to demonstrate any significant difference here even though the tendency is unchanged.

(3) Finally the efficacy of the treatment has been evaluated from the duration of the first and second stages of labour as can be seen from Table IX. Here it can be seen that the first stage is significantly shorter ($p < 0.05$) for the patients of ODA group than for the SYT group of patients. If the patients are divided into two groups according to the indication for treatment it is also found that the average first stage of labour is shorter for the ODA group patients than for the SYT group—both after induction of labour and stimulation of labour but a significant difference between the results is only found after the stimulation of labour

Table IX. *The efficacy of the treatment evaluated from the duration of the 1st and 2nd stages of labour with successful attempts*

	SYT	ODA
Dilatation time in minutes (1st stage)	374±265	301±239
Expulsion time in minutes (2nd stage)	25±17	28±2_

The first stage of labour in the two groups has also been evaluated in relation to the pelvic score at the start of the treatment. A shorter first stage was also found here for patients treated with ODA but in neither one was it possible to find any significant difference between the results in the two treatment groups.

Finally the second stage of labour was compared in the two groups. No significant difference was noted in the results in the two treatment groups.

The absolute figures for the complications in connection with the successful attempts at treatment are shown in Table X. The figures are so small that no conclusions can be drawn. The frequency of complications is estimated to be of the same magnitude in both groups. This confirms the findings of other similar studies (7).

Table X *Complications after successful attempts at treatment*

	SYT (103 pat.)	ODA (87 pat.)
Dead child	0	0
Apgar score less than 7	3	2
Retained placenta	3	3
Haemorrhage (500 ml) during birth	1	1

No other side-effects of the treatment have been recorded. In particular neither the induction nor stimulation of labour gave rise to such hypertonic uterine activity that treatment had to be discontinued.

CONCLUSION

No statistically significant difference can be demonstrated between the results of induction or stimulation of labour with oxytocin and desaminooxytocin, as evaluated by the total number of successful attempts at treatment. On the other hand it is found that the dosage of biologically active compound, expressed in international units, required to achieve delivery is significantly lower in the ODA group as a whole. Even when regard is taken of the greater biological effect of ODA on the human uterus, there is a statistically significant difference ($p < 0.01$). Finally it is found that the first stage of labour is significantly shorter for patients treated with desaminooxytocin even though this difference only becomes evident following comparison of the groups of patients in whom labour has been stimulated. The difference is not significant for the patients in whom labour was induced.

Side-effects and birth complications were so few and slight in both treatment groups that no treatment had to be stopped because of them.

REFERENCES

1. Berde, B. & Saameli, K. Evaluation of substances acting on the uterus. Brit Pharmacol Soc. Summer Meeting, Dublin 10-12 07 1963.
2. Berger, M. Transbuccale Anwendung von Oxytocin und eines Oxytocin-Derivates. Praxis 57 588-594, 1968.
3. Bishop, E. H. Pelvic scoring for elective induction. Obstet Gynaec 24 266-68, 1964.
4. Chan, W. Y. & Vigneaud, V. Comparison of the properties of oxytocin and its highly potent analogue, desaminooxytocin. Endocrinology 71 977 1962.
5. Du Vigneaud, V. et al. Synthesis of 1-B-mercaptopropionic acid oxytocin (desaminooxytocin), highly potent analogue of oxytocin. J Biol Chem 235 64-66, 1960.
6. Embrey, M. P. The action of desamino-oxytocin on the human pregnant uterus. Endocrinology 31 183 1965.
7. Golobow, J. et al. Effect of human pregnancy serum on avian depressor activities of oxytocin and desamino-oxytocin. Proc Soc Exper Bio Med 113 113-115 1963.
8. Jansson, I. Effect of desamino-oxytocin on the human pregnant uterus at term. Acta Obstet Gynec Scand 14 29-42, 1966.
9. Masd, G. B. et al. Effetti della desamino-ossitocina sulla contrattilità del utero umano a termine di gravidanza. Riv Obstet Ginec 20 77-86, 1965.
10. Obolensky, W. The influence of buccal desaminooxytocin on the uterus and the fetus. Int J Gynaec Obstet 8, 2/2: 160, 1970.
11. Rebsteiner, H. P. Induction of labour with buccal tablets of syntocinon and desamino-oxytocin. Int J Gynec Obstet 8, 2/2 160, 1970.
12. Senn, K. Desamino-Oxytocin, ein von Schwangerenblut nicht inaktiviertes Wehen-Hormon. Geburtsh Frauenheilk 27 36-42, 1967.

Submitted for publication Aug. 8 1972

Erik Fangel Poulsen
St Thomas Allé 2
1824 Copenhagen
Denmark

Acta Obstet Gynec Scand 52, 309–315 1973

THE EFFECT OF OXYGEN VENTILATION AND A VASODILATOR ON UTERINE PERFUSION FETAL OXYGEN AND ACID-BASE BALANCE

I. *A Study in Abnormal Gravidae*

Leo Tervilä, Eero Vartiainen, Inkeri Kivalo, Matts-Johan Fimnilä
and Martti Väyrynen

*From Departments I and II of Obstetrics and Gynaecology, University Central Hospital
(Heads: Prof. S. Timonen and Prof. P. Vara), Helsinki, Finland*

Abstract. The effect of oxygen and xantinol nicotinate (Complamin®) alone or in conjunction on uterine perfusion and the acid-base balance and p_{O_2} of the fetal blood was studied in gravidae suffering from various diseases. The series consisted of 40 gravidae with hypertension and 32 suffering from other diseases. Oxygen ventilation had an unfavourable effect on uterine perfusion in the latter group, just as was found in normal gravidae in a previous study. A still greater depression of uterine perfusion was observed in the hypertensive patients. The fetal p_{O_2} rose slightly and the fetal pH fell during oxygen ventilation. Fetal BE and p_{CO_2} showed slight rises. Uterine perfusion was also slightly depressed by Complamin® addition. On the other hand no appreciable changes were noted in the fetal blood chemistry. Oxygen and Complamin® in conjunction caused a depression of perfusion similar to that observed with oxygen alone. The response of the fetal pH was significantly more favourable in the combined group than in the oxygen group, and p_{O_2} rose, just as in the oxygen group. The conclusion may be drawn that oxygen ventilation of the mother at the final stage of gestation has an unfavourable effect on the fetal acid-base balance. It seems possible that this effect is to some extent counteracted by Complamin® administration in conjunction with oxygen ventilation.

Certain diseases during pregnancy obviously affect uterine circulation. Hypertension, in particular, has an unfavourable effect (1 4 5). Histological changes correlating with the severity of the disorder have been observed in the uterine vasculature (2 3 7). It thus seems possible that the changes in uterine perfusion caused by oxygen ventilation and drug intake are different in gravidae with hypertension or other diseases as compared with normal gravidae.

In order to study this question the changes in uterine perfusion and fetal blood chemistry were measured in gravidae with hypertension and certain other diseases. The results were compared to those obtained in a series of normal gravidae (6).

MATERIAL

The series consisted of 64 gravidae at the 36th or subsequent weeks of pregnancy and 8 in the 32nd–36th weeks. The former group comprised 40 women with a blood pressure exceeding 140/90. Among these there were 8 with blood pressure over 175/115. In addition, the series included 7 patients with haemoglobin value under 11 g/100 ml, 4 diabetics, 4 cases of Rh incompatibility 5 patients with hepatogestosis, 2 with fetal asphyxia and one with proteinuria.

METHODS

Of the patients with hypertension 18 were given 100% oxygen by mask with an anaesthesia machine using semiclosed system and CO_2 absorber, 10 received 300 mg Complamin® (xantinol nicotinate), 5 were given oxygen and Complamin® in conjunction, and 7 were given 50% oxygen-nitrogen mixture. Of the anaemic patients 3 received oxygen and 4 Complamin®. The diabetics and the patients with Rh incompatibility received oxygen alone. Of the patients with hepatogestosis one was given oxygen, while the remaining 4 received Complamin®. The patient with proteinuria was given Complamin®. The patients with fetal asphyxia were all air-ventilated by mask. Of the patients with premature deliveries half were given oxygen, half Complamin®. Uterine perfusion was measured in all patients with a thermistor-needle inserted into the portio. Blood samples were drawn from the portio

Xantinol nicotinate (Complamin®) used in this study was kindly supplied by Johann A. Wülfing, Düsseldorf, Western-Germany

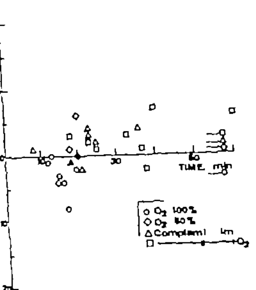


Fig 5 Changes in p_{O_2} in capillary blood from the fetal scalp

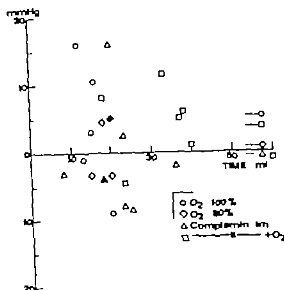


Fig 7 Changes in p_{CO_2} in capillary blood from the fetal scalp.

rise was clearly lower in those given 50% oxygen than in those who received 100% oxygen. The ... between the rise in fetal, portio and ear bloods was about 1 37 120. Complamin® alone caused no change in p_{O_2}. I the combined group a rise in fetal p_{O_2} was noted, but compared to the other groups the difference was not statistically significant

As regards the changes in p_H an inverse correlation was noted in the hypertensive group inasmuch as oxygen alone caused the greatest decrease. The decrease was even greater in fetal blood than in portio blood. In the combined group the fetal value improved to a significantly greater extent compared to the oxygen group ($p<0.01$). Complamin® alone had a slightly more

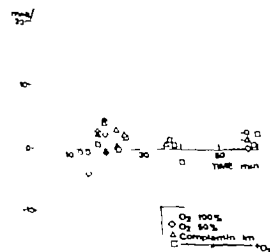


Fig ... Changes in BE in capillary blood from the fetal scalp

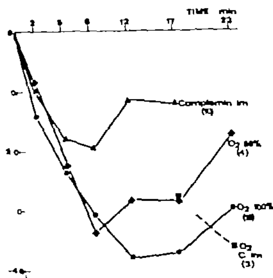


Fig 8 Uterine perfusion in hypertensive patients. No. of cases in brackets.

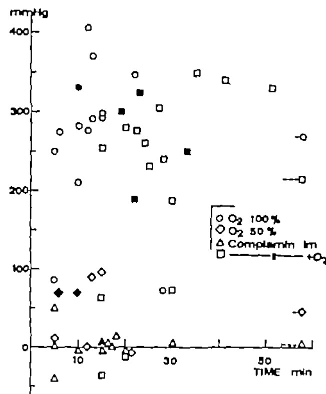


Fig 1 Changes in p_{O_2} in capillary blood from the ear f hypertensive patients. Closed signs show the cases with a blood pressure over 175/115 mmHg. The averages on the right.

and the ear of the mother nd the scalp of the fetus. The p_{O_2} was determined in all samples. In add tion, pH was determined in portio blood and fetal blood. The

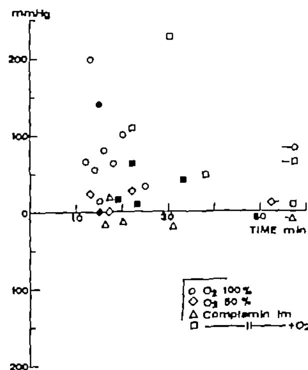


Fig 2 Changes i p_{O_2} in capillary blood from the portio.

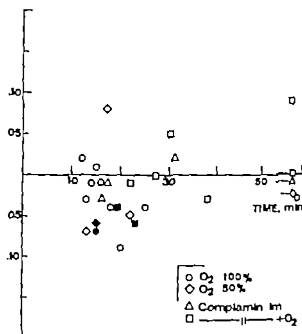


Fig 3 Changes in pH in capillary blood from the portio.

fetal samples wer also used for determination of BE and p_{CO_2}.

For details of the method the reader is referred to a previous report (6)

RESULTS

The blood values obtained in the patients with hypertension are given in Figs 1–7 The p_{O_2} rose throughout in all oxygen-ventilated patients. The

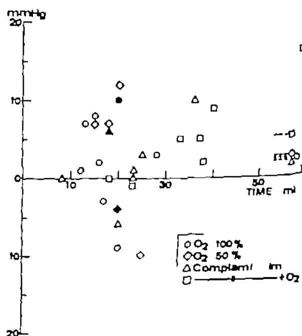


Fig 4 Changes n po m capillary blood from the fetal scalp.

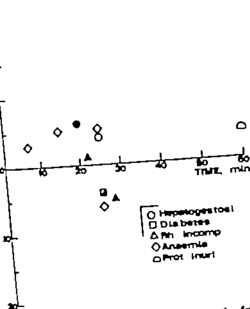


Fig. 11 Changes in p_H in capillary blood from the fetal scalp. For the signs see Fig. 9

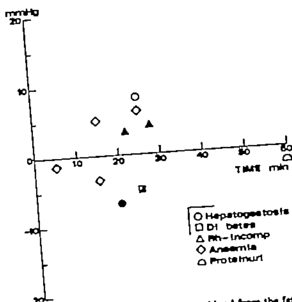


Fig. 13 Changes in p_{CO_2} in capillary blood from the fetal scalp. For the signs see Fig. 9

In the group of other diseases the results did not attain the level of significance owing to the scanty material. The values may be seen in Figs. 9–15. Similar changes were observed as in the hypertensive patients, although the effect of oxygen ventilation on fetal p_{O_2} seemed to be somewhat more favourable. The changes in p_H were perhaps slightly more favourable in the Complamin® group than in the oxygen group. Oxygen had no appreciable effect on fetal BE and p_{CO_2}, while Complamin® caused a slight rise of these values.

The changes in uterine perfusion are shown in Fig. 16, which also demonstrates the marked

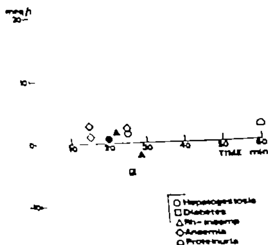


Fig. 1 Changes in BE in capillary blood from the fetal scalp. For the signs see Fig. 9

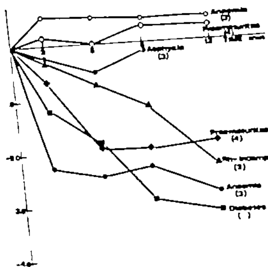


Fig. 16. Uterine perfusion in patients with various diseases. No. of cases in brackets. Closed signs show the cases given 100% O_2, half-closed air and open signs Complamin®

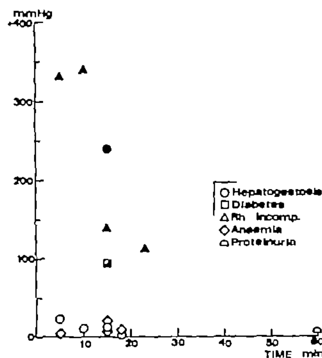


Fig 9 Changes in p_{O_2} in capillary blood from the ear of patients with various diseases. Closed signs show the cases given 100% O_2, half-closed 50% O_2 and open signs Complamin®

favourable effect than oxygen ($p < 0.05$). Fetal BE and p_{CO_2} both seemed to rise in the 100% oxygen group, although no statistically significant changes were observed.

Oxygen ventilation depressed uterine perfusion

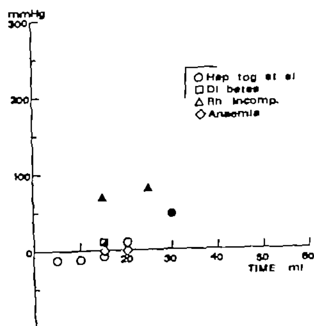


Fig 10 Changes in p_{O_2} in capillary blood from the portio. For the signs see Fig. 9

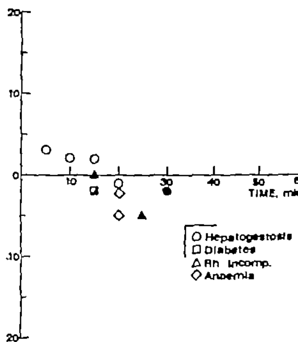


Fig 11 Changes in p_H in capillary blood from the portio. For the signs see Fig. 9

the most (Fig. 8). The difference between the oxygen group and the Complamin® group was almost significant ($p < 0.05$). In the combined group measurements mostly failed during the first 12 min of the observation time. The values obtained at 17 and 23 min were of the same order as in the oxygen group.

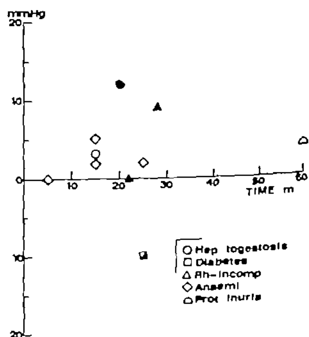


Fig 12. Changes in p_{O_2} in capillary blood from the fetal scalp. For the signs see Fig 9

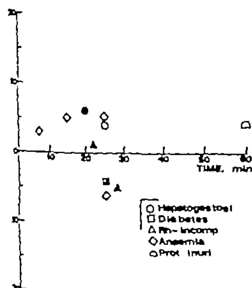


Fig. 11 Changes in p_{O_2} in capillary blood from the fetal scalp. For the signs see Fig. 9.

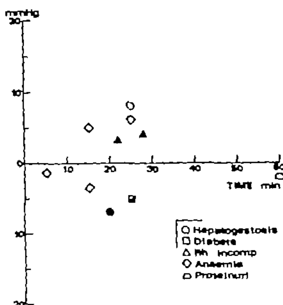


Fig. 13 Changes in p_{CO_2} in capillary blood from the fetal scalp. For the signs see Fig. 9

In the group of other diseases the results did not attain the level of significance owing to the scanty material. The values may be seen in Figs. 9-15. Similar changes were observed as in the hypertensive patients, although the effect of oxygen ventilation on fetal p_{O_2} seemed to be somewhat more favourable. The changes in p_H were perhaps slightly more favourable in the Complamin® group than in the oxygen group. Oxygen had no appreciable effect on fetal BE and p_{CO_2} while Complamin® caused a slight rise of these values.

The changes in uterine perfusion are shown in Fig. 16, which also demonstrates the marked

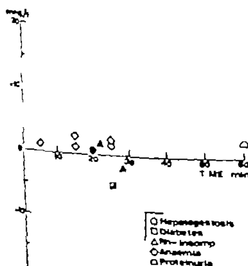


Fig. 1 Changes in BE in capillary blood from the fetal scalp. For the signs see Fig. 9

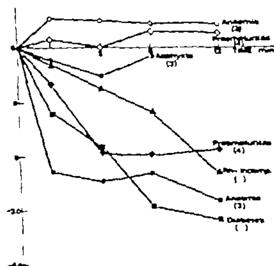


Fig. 16 Uterine perfusion in patients with various diseases. No. of cases in brackets. Closed signs show the cases given 100% O_2, half-closed air and open signs Complamin®.

depression of the portio circulation caused by oxygen ventilation On the other hand in the 7 patients given Complamin® perfusion improved during the observation time. This was the only group in which an improvement was observed. This group consisted of 3 patients with anaemia and 4 with premature deliveries. In 3 patients air ventilated by mask perfusion fell slightly only to return to the previous level within 8 min.

DISCUSSION

Oxygen ventilation caused an obvious depression of uterine perfusion in gravidae with hypertension and certain other diseases associated with pregnancy This depression was clearly greater in the hypertensive patients than in those suffering from other diseases and in normal gravidae (6) This finding is surprising, considering that obvious morphological changes observed in the uterine vasculature of hypertensive gravidae seem to indicate that the uterine vascular walls are less elastic than in normal subjects (3) The tendency towards capillary spasm associated with hypertension thus seems to compensate this disadvantage.

A similar observation was made with regard to the changes in fetal p_{O_2}, inasmuch as a rise of the same order was noted in normal gravidae and in patients with diseases other than hypertension, while a lesser rise was observed in hypertensive gravidae By contrast, a greater impairment of the fetal p_H was observed in the hypertensive group

When the changes in fetal p_H in the Complamin® groups and the oxygen group were compared, significant differences were noted. Complamin® given both alone and in conjunction with oxygen had a clearly more favourable effect on the fetal p_H This difference was more marked in the hypertensive patients than in normal gravidae. It thus seems possible that Complamin® has a slight effect on the constricted capillaries in hypertension which is not manifested in normal subjects.

The present investigation clearly shows that oxygen ventilation of the mother has a depressing effect on the p_H of the fetal blood, although this effect is slight. However an associated, obvious rise of the fetal p_{O_2} was also observed. The observation time was obviously too short for signs of metabolic acidosis to develop in any of the test groups.

Although Complamin® given alone in some cases seemed to have a favourable effect on uterine perfusion, the fetal chemical blood values were not improved. When the drug is administered in conjunction with oxygen it may to some extent counteract the untoward effects of oxygen, particularly in hypertensive patients. The effect is only of the order of a 0.02–0.03 rise of the p_H but in conjunction with a slightly increased p_{O_2} this may be of some importance for the condition of the fetus, as it implies an increase in the oxygen saturation of the fetal blood Because fetal haemoglobin was not measured we could not determine actual quantitative changes in fetal oxygen saturation.

The perfusion curve fell for one or two minutes after insertion of the thermistor-needle. Since this drop was also noted in the air ventilated group, it was obviously due to capillary spasm resulting from the irritation caused by the procedure. The true depression of perfusion in the test groups is thus obtained by subtracting this apparent decrease from the results. The phenomenon in question is, however of no practical consequence, since the values representing perfusion obtained in this investigation are relative and only demonstrate differences between groups.

REFERENCES

1 Browne, J C. McClure & Veall, N. The maternal placental blood flow in normotensive and hypertensive women. J Obstet Gynaec Brit Emp 60, 141 1953.

2 Burstein, R., Blumenthal, H T & Soule S. D Histogenesis of pathological processes in placentas of metabolic disease in pregnancy I. Toxemia and hypertension. Am J Obstet Gynec 74 85 1957

3 Dixon, H G & Robertson, W B. A study of the vessels of the placental bed in normotensive and hypertensive women. J Obstet Gynaec Brit Emp 65 803, 1958

4 Kuma D Chronic placental ischemia in relation to toxemias of pregnancy Am J Obstet Gynaec 84 1323, 1962.

5 Morris, N Osborn, S B. & Wright, H. P Effective circulation of the uterine wall in late pregnancy measured with [24]NaCl Lancet I 323 1955

6 T[illegible], L V[illegible], E Kivalo L, Frenell, M J &

Väyrynen, M. The effect of oxygen ventilation and vasodilatator on uterine perfusion and fetal oxygen and acid-base balance. I. A study with healthy gravidas. Acta Obstet Gynec Scand, 52: 175, 1973.

7 Zeek, P. M. & Assali, N. S.: Vascular changes in the decidua associated with eclamptogenic toxemia of pregnancy. Am J Clin Pathol 20: 1099, 1950.

Submitted for publication Sept. 24, 1972

Leo Tervilä
Department of Obstetrics and Gynaecology
University Central Hospital
Helsinki
Finland

depression of the portio circulation caused by oxygen ventilation On the other hand in the 7 patients given Complamin® perfusion improved during the observation time This was the only group in which an improvement was observed. This group consisted of 3 patients with anaemia and 4 with premature deliveries. In 3 patients air ventilated by mask perfusion fell slightly only to return to the previous level within 8 min.

DISCUSSION

Oxygen ventilation caused an obvious depression of uterine perfusion in gravidae with hypertension and certain other diseases associated with pregnancy This depression was clearly greater in the hypertensive patients than in those suffering from other diseases and in normal gravidae (6). This finding is surprising, considering that obvious morphological changes observed in the uterine vasculature of hypertensive gravidae seem to indicate that the uterine vascular walls are less elastic than in normal subjects (3) The tendency towards capillary spasm associated with hypertension thus seems to compensate this disadvantage.

A similar observation was made with regard to the changes in fetal p_{O_2} inasmuch as a rise of the same order was noted in normal gravidae and in patients with diseases other than hypertension while a lesser rise was observed in hypertensive gravidae. By contrast a greater impairment of the fetal p_H was observed in the hypertensive group.

When the changes in fetal p_H in the Complamin® groups and the oxygen group were compared, significant differences were noted Complamin® given both alone and in conjunction with oxygen had a clearly more favourable effect on the fetal p_H This difference was more marked in the hypertensive patients than in normal gravidae. It thus seems possible that Complamin® has a slight effect on the constricted capillaries in hypertension which is not manifested in normal subjects.

The present investigation clearly shows that oxygen ventilation of the mother has a depressing effect on the p_H of the fetal blood although this effect is slight. However an associated obvious rise of the fetal p_{O_2} was also observed. The observation time was obviously too short for signs of metabolic acidosis to develop in any of the test groups.

Although Complamin® given alone in some cases seemed to have a favourable effect on uterine perfusion the fetal chemical blood values were not improved. When the drug is administered in conjunction with oxygen it may to some extent counteract the untoward effects of oxygen, particularly in hypertensive patients. The effect is only of the order of a 0.02–0.03 rise of the p_H but in conjunction with a slightly increased p_{O_2} this may be of some importance for the condition of the fetus, as it implies an increase in the oxygen saturation of the fetal blood. Because fetal haemoglobin was not measured we could not determine actual quantitative changes in fetal oxygen saturation.

The perfusion curve fell for one or two minutes after insertion of the thermistor-needle. Since this drop was also noted in the air-ventilated group, it was obviously due to capillary spasm resulting from the irritation caused by the procedure The true depression of perfusion in the test groups is thus obtained by subtracting this apparent decrease from the results. The phenomenon in question is, however of no practical consequence since the values representing perfusion obtained in this investigation are relative and only demonstrate differences between groups.

REFERENCES

1 Browne J C McClure & Veall, N The maternal placental blood flow in normotensive and hypertensive women J Obstet Gynaec Brit Emp 60 141 1953

2. Burstein, R., Blumenthal, H. T & Soule S D Histogenesis of pathological processes in placentas of metabolic disease in pregnancy I. Toxemia and hypertension. Am J Obstet Gynec 74 85 1957

3 Dixon, H. G & Robertson, W. B A study of the vessels of the placental bed in normotensive and hypertensive women. J Obstet Gynaec Brit Emp 65 803, 1958.

4. Kumar D Chronic placental ischemia in relation to toxemias of pregnancy Am J Obstet Gynec 84 1323 196_

5 Morris, N Osborn, S B. & Wright, H. P Effective circulation of the uterine wall in late pregnancy measured with [24]NaCl Lancet 1 323, 1955

6 Teramo, L V [illegible], E [illegible], J [illegible], M J &

Acta Obstet Gynec Scand 52, 317–3.1 1973

MEASUREMENT OF UTERINE BLOOD FLOW IN NON-PREGNANT WOMEN BY ELECTROMAGNETIC FLOWMETER

Effect of oxytocin

Inge Klingenberg

From the Department of Obstetrics and Gynaecology (Head, T. Dahle, M.D.), Ullevål Hospital and the Institute for Experimental Medical Research, University of Oslo (Head, F. Kiil, M.D.), Ullevål Hospital, Oslo, Norway

Abstract Blood flow in one uterine artery was measured by the electromagnetic flowmeter method in non-pregnant women undergoing surgery. Due to difficulties in dissecting and exposing the uterine artery, satisfactory signals for measuring blood flow were obtained in only 10 out of 22 patients. Most of them had uterine myomata. Mean blood flow in one uterine artery was found to be 51.5 ml/min. After injection of oxytocin blood flow was reduced to 22 to 75% of controls, except in one patient, where no flow change was observed. Methergin administered in 3 patients caused no changes in uterine blood flow.

Numerous observations have been reported on the effect of oxytocin on human uterine motility while information about its effect on human uterine blood flow has been limited due to a lack of suitable methods.

When Kolin (11) and Wetterer (12) developed electromagnetic flowmeters, they introduced a method which was well fitted for recording changes in arterial blood flow if the vessel could be freed from its adventitial connections for a length of one to three centimetres. Later the electromagnetic flowmeter has often been used for uterine blood flow measurements in animals, but only one study (3) seems to have been made in the human.

In order to obtain information about the magnitude of human uterine arterial blood flow and to study the effect of oxytocin on it, measurements were carried out on one uterine artery by the electromagnetic flowmeter method under general anaesthesia. The influence of ergometrine was also recorded in some cases.

METHODS

Blood flow in the left or right human uterine artery was measured by an electromagnetic flowmeter, Nycotron, Type 372-M.

Probes with a diameter of 2.5 and 3 mm were calibrated in vitro on human uterine arteries in saline.

The drugs used were oxytocin (Pitocin, "Parke, Davis & Company") and methylergometrine maleate (Methergin, "Sandoz").

Observations were made in patients during gynaecological laparotomies under general anaesthesia (Hexobarbital, Succinylcholine, Nitrous Oxide, Halothane).

The anterior layer of the broad ligament was opened, and the uterine artery was exposed and freed from surrounding tissue for a length of about 3 cm. The artery was then slipped into the gap of the probe, and the probe fixed in a position which did not displace the vessel. In some cases the dissection and the difficulties of bringing the artery into the gap of the probe involved much manipulation of the vessel, especially when there were pelvic adhesions or when the vessels were tortuous or adherent to each other. In order to provide good contact between the vessel and probe, the space formed by the two layers of the opened broad ligament was filled with saline solution to above the level of the artery and the probe. A catgut thread was placed loosely around the uterine artery to stop the blood flow and enable the flowmeter to be adjusted to zero. Both ends of catgut were fixed into the opening of a Deschamps needle, and the artery was pressed against the instrument by tightening the catgut. This procedure was performed before and after the measurements.

The drugs were given by a permanent intravenous drip in the arm and arterial blood pressure was recorded at intervals by the cuff method.

MATERIAL

The study was carried out on 10 women (Table I) undergoing laparotomies for benign gynaecological diseases. A

Table II. *Blood flow in one uterine artery before and after injection of oxytocin*

				Before oxytocin injection					After oxytocin injection				
									Blood flow				
Pat. no	Age (y)	Day of menstr cycle	Endometrial phase	Blood flow (ml/min)	Blood pressure (mmHg)	Pulse rate	Uterine vascular resistance	Dose (IU)	(ml/min)	Per cent of flow before oxytocin	Blood pressure (mmHg)	Pulse rate	Uterine vascular resistance
2	50	—	—	43	120/70	88	2.21	5	30	70	120/70	88	3.17
3	43	3	Proliferative	69	115/80	100	1.33	5	48	70	115/65	120	1.71
4	51	7	Proliferative	61	140/110	100	1.97	5	45	74	120/85	112	2.16
5	48	—	Prol./secr.	29	80/60	92	2.41	5	16	55	80/60	102	4.38
6	31	25	Secretory	77	100/70	66	1.12	5	77	100	100/70	84	1.12
7	43	27	Secretory	63	105/80	70	1.44	10	14	22	80/50	88	4.28

Oxytocin 5 IU was injected into the intravenous drip over a 10 second interval (Fig. 1 arrow). About 18 sec later the blood flow began to decrease, the diastolic flow more pronounced than the systolic, and after a further 24 sec the curve reached a steady level, at which it remained until the end of the experiment 3 min later.

Constriction of the uterine artery was often observed during dissection. When the probe had been placed around the artery, blood flow usually rose in the first 2 to 5 min. Then a steady state was obtained and this value was recorded as control flow.

As shown in Table II, oxytocin was given intravenously to six patients, five received 5 IU and one 10 IU. Blanching of the uterine surface was observed after oxytocin injection in some cases. The injection time lasted from 5 to 20 sec. A fall in blood flow occurred within the first minute after injection. Uterine blood flow decreased after injection in five patients to 22 to 74% of controls. At the time blood flow was recorded after oxytocin administration, blood pressure was found to be unchanged in two of the patients and decreased in three. Uterine vascular resistance (mean blood pressure divided by mean uterine blood flow) was calculated for the patients before and after oxytocin injection and showed an increased resistance. One patient (No. 6), who had received 5 IU oxytocin, showed no changes in uterine arterial blood flow and brachial blood pressure.

Methergin, 0.1 mg was given intravenously to 3 patients. No change in uterine blood flow occurred during the observation time of 3–4 min and blood pressure remained constant.

DISCUSSION

The main difficulties involved in the use of an electromagnetic flowmeter were the dissection and the application of the probe to the uterine artery without excessive manipulation and bleeding. About half of the attempted measurements were unsuccessful because of these problems. Before beginning dissection, the pulse in the uterine artery could always be palpated in these cases, but after the dissection or after the artery was slipped into the gap of the probe no pulse could be felt. It is likely that the same tendency was present in all patients and this assumption—together with the observed constriction of the arteries during dissection—indicates that the recorded flow values are minimal.

The increase in uterine weight above normal was mainly due to myomas. Arterial blood flow was of the same magnitude in the normal sized uterus (No. 6) as in the uteri with myomas. The heavier uteri showed no increase in arterial blood flow. This suggests a lower blood flow in the myoma than in the myometrium, which is consistent with the paucity of blood vessels in myomas compared with normal myometrial tissue. However, absolute blood flow in the myometrium and in the myomas cannot directly be estimated from the present results.

If the assumptions are made that both uterine arteries carry the same blood supply to uterus and comprise the major part of uterine flow, the mean flow to the whole uterus in the present series was about 100 ml/min, or about ? % of cardiac output in non-pregnant women. This is in agreement with the local uterine blood flow obtained

Table I *Blood flow measurements in one uterine artery*

Pat. no.	Age (y)	Diagnosis	Nos. of pregnancies	Day of menstr cycle	Endometrial phase	Uterine weight (g)	Blood flow (ml/min)
1	42	Uterine myomas	0	20	Secretory	500	40
2	50	Uterine myomas	2	—		350	43
3	43	Uterine myomas	3	3	Proliferative	470	69
4	51	Uterine myomas	1	7	Proliferative	420	61
5	48	Uterine myomas	1	—	Prol/secr	250	79
6	38	Resection of the Fallopian tubes	4	25	Secretory	About[b] 100	77
7	43	Uterine myomas		27	Secretory	350	63
8	43	Uterine myomas	3	19	Late prol.	240	46
9	44	Uterine myomas	2	—	Secretory	175	70[a]
10	49	Uterine myomas	5	10	Proliferative	1 000	29[a]

Measurements performed on the ascending branch of the uterine artery
[b] The uterine weight for patient 6 was estimated at about 100 g.

further twelve patients had to be excluded from the series because of surgical or methodological difficulties. The age of the patients ranged from 38 to 51 years. Nine patients suffered from uterine bleeding or myomas and had a hysterectomy performed. The removed uteri were weighed. In patient 6 the Fallopian tubes were resected.

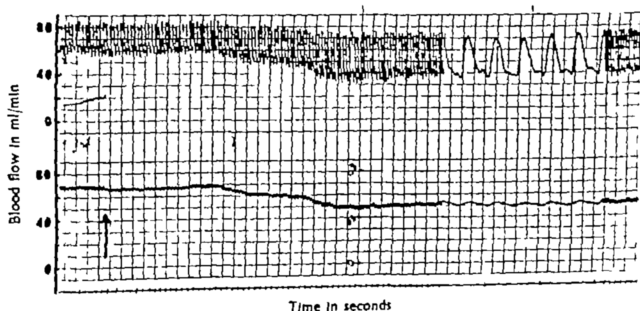
RESULTS

Satisfactory flow measurements were made on the uterine artery in 8 patients and on the ascending branch of the uterine artery in 2 patients.

Table I presents a survey of the measurements in the 10 patients. Blood flow varied between 29 and 77 *ml/min with a mean of 53 5 ml/min. In* patients 9 and 10 the probe was applied on the ascending limb of the uterine artery and these measurements were therefore excluded from the calculation of the mean flow

Blood flow in the uterine artery showed no obvious relationship to parity menstrual phase or weight of the uterus.

Fig 1 demonstrates flow recording on the right uterine artery in patient 3 The upper curve shows pulsatile blood flow the lower curve mean flow obtained at recording rates of 2.5 and 25 mm/sec.



I Effect of oxytocin on blood flow in the uterine 3 Upper curve shows pulsatile flow lower curve mean flow Oxytocin 5 IU as injected into the intra enous d p the arm at the arrow

Table II *Blood flow in one uterine artery before and after injection of oxytocin*

				Before oxytocin injection					After oxytocin injection				
									Blood flow				
Pat. no	Age (y)	Day of menstr cycle	Endometrial phase	Blood flow (ml/min)	Blood pressure (mmHg)	Pulse rate	Uterine vascular resistance	Dose (IU)	(Ml/min)	In per cent of flow before oxytocin	Blood pressure (mmHg)	Pulse rate	Uterine vascular resistance
[illegible]	50	—	—	43	120/70	88	2.21	5	30	70	120/70	88	3.17
[illegible]	43	3	Proliferative	69	115 80	100	1.33	5	48	70	115 65	120	1 71
[illegible]	51	7	Proliferative	61	140 110	100	1.97	5	45	74	120 85	112	2 16
[illegible]	48	—	Prol /secr	29	80 60	92	2 41	5	16	55	80,60	102	4.38
[illegible]	38	25	Secretory	77	100/70	64	1 12	5	77	100	100/70	84	1 12
[illegible]	43	27	Secretory	63	105/80	70	1 46	10	14	22	80/50	88	4 28

Oxytocin 5 IU was injected into the intravenous drip over a 10 second interval (Fig. 1 arrow). About 18 sec later the blood flow began to decrease, the diastolic flow more pronounced than the systolic and after a further 24 sec the curve reached a steady level at which it remained until the end of the experiment 3 min later

Constriction of the uterine artery was often observed during dissection. When the probe had been placed around the artery blood flow usually rose in the first 2 to 5 min Then a steady state was obtained and this value was recorded as control flow

As shown in Table II oxytocin was given intravenously to six patients, five received 5 IU and one 10 IU Blanching of the uterine surface was observed after oxytocin injection in some cases. The injection time lasted from 5 to 20 sec A fall in blood flow occurred within the first minute after injection Uterine blood flow decreased after injection in five patients to 22 to 74 of controls At the time blood flow was recorded after oxytocin administration, blood pressure as found to be unchanged in tw of the patients and decreased in three Uterine ascular resistance (mean blood pressure divided by mean uterine blood flow) was calculated for the patients before and after oxytocin inject n and showed an increased resistance One patient (No 6), ho had received 5 IU oxytocin show d no changes in uterine arterial blood flow and brachial blood pressure

Methergin 0 1 mg was given intravenously to 3 patients No change in terine blood flow oc urred during the observation time of 3–4 min and bl d pressure remained constant

DISCUSSION

The main difficulties involved in the use of an electromagnetic flowmeter were the dissection and the application of the probe to the uterine artery without excessive manipulation and bleeding. About half of the attempted measurements were unsuccessful because of these problems. Before beginning dissection the pulse in the uterine artery could always be palpated in these cases but after the dissection or after the artery was slipped into the gap of the probe, no pulse could be felt. It is likely that the same tendency was present in ll patients and this assumption—together with the observed constriction of the arteries during dissection—indicates that the recorded flow values are minimal

The increase in uterine weight above normal wa mainly due to myomas. Arterial blood flow was of the same magnitude in the normal sized uterus (No 6) as in the uteri with myomas The heavier uteri showed no increase in arterial blood flow This suggest a lowe blood flow in the myomas than in the myometrium, which is consistent with the paucity of blood essels in myoma compared w th normal myometrial tissue How ever absolute blood flow in the myometrium and in the myomas cannot directly be estimated from the present results.

If the assumptions are made that both terine arteries carry the same blood supply to uterus and comprise the major part of uterine flow the mean flow to the whole uterus in the present series was about 100 ml min or about of cardiac output in non-pregnant women This is in agreement th the local uterine blood flow obtained

Table I *Blood flow measurements in one uterine artery*

Pat. no	Age (y)	Diagnosis	Nos. of pregnancies	Day of menstr cycle	Endometrial phase	Uterine weight (g)	Blood flow (ml/min)
1	42	Uterine myomas	0	20	Secretory	500	40
2	50	Uterine myomas	2	—		350	43
3	43	Uterine myomas	1	3	Proliferative	420	69
4	51	Uterine myomas	1	7	Proliferative	420	61
5	48	Uterine myomas	1	—	Prol/secr	250	29
6	38	Resection of the Fallopian tubes	4	25	Secretory	About[b] 100	77
7	43	Uterine myomas	2	27	Secretory	350	63
8	43	Uterine myomas	3	19	Late prol.	240	46
9	44	Uterine myomas	2	—	Secretory	175	20[a]
10	49	Uterine myomas	5	10	Proliferative	1 000	79[a]

[a] Measurements performed on the ascending branch of the uterine artery
[b] The uterine weight for patient 6 was estimated at about 100 g.

further twelve patients had to be excluded from the series because of surgical or methodological difficulties. The age of the patients ranged from 38 to 51 years. Nine patients suffered from uterine bleeding or myomas and had a hysterectomy performed. The removed uteri were weighed. In patient 6 the Fallopian tubes were resected.

RESULTS

Satisfactory flow measurements were made on the uterine artery in 8 patients and on the ascending branch of the uterine artery in 2 patients.

Table I presents a survey of the measurements in the 10 patients. Blood flow varied between 29 and 77 ml/min with a mean of 53.5 ml/min. In patients 9 and 10 the probe was applied on the ascending limb of the uterine artery and these measurements were therefore excluded from the calculation of the mean flow.

Blood flow in the uterine artery showed no obvious relationship to parity, menstrual phase or weight of the uterus.

Fig. 1 demonstrates flow recording on the right uterine artery in patient 3. The upper curve shows pulsatile blood flow, the lower curve mean flow obtained at recording rates of 2.5 and 25 mm/sec.

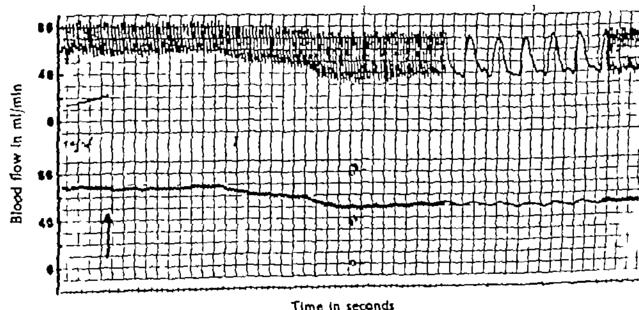


Fig. 1. Effect of oxytocin on blood flow in the uterine artery in patient 3. Upper curve shows pulsatile flow, lower curve mean flow. Oxytocin 5 IU was injected into the intravenous drip in the arm at the arrow.

VI. Effects of oxytocic, vasopressor and vasodepressor drugs on the blood flow to the postpartum uterus in unanesthetized sheep. Amer J Obstet Gynec 78 313–321, 1959.
3. Assali, N. S., Rauramo, L. & Peltonen, T. Measurement of uterine blood flow and uterine metabolism. VIII. Uterine and fetal blood flow and oxygen consumption in early human pregnancy. Amer J Obstet Gynec 79 86–98, 1960.
4. Dilts, P. V. Jr, Kirschbaum, T. H. & Assali, N. S. 1967. Quoted from Biology of Gestation (ed. N. S. Assali), vol. I, p. 192, 1968.
5. Greiss, F. C. Effect of labor on uterine blood flow. Amer J Obstet Gynec 93 917–923, 1965.
6. Jansson, I.: 133Xenon clearance in the myometrium of pregnant and non-pregnant women. Acta Obstet Gynec Scand 48:302–321, 1969.
7. Kalman, S. M. The effect of estrogens on uterine blood flow in the rat. J Pharmacol Exp Ther 124 179–181, 1958.
8. Kitchin, A. H., Lloyd, Sybil M. & Pickford, Mary. Some actions of oxytocin on the cardiovascular system in man. Clin Sci 18 399–406, 1959.
9. Klingenberg, I. Measurement of blood flow in human myometrium by local hydrogen clearance. Acta Obstet Gynec Scand 48 470–478, 1969.
10. — The effect of oxytocin on human uterine blood flow measured by local hydrogen clearance. In press.
11. Kolin, A. An electromagnetic flowmeter. Principle of the method and its application to bloodflow measurements. Proc Soc Exp Biol Med 35 53–56, 1936.
12. Wetterer, E.: Eine neue Methode zur Registrierung der Blutströmungsgeschwindigkeit am unereöffneten Gefäss. Z Biol 98 26–36, 1937.

Submitted for publication Oct. 10, 1972

Inge Klingenberg
Dept. of Obstetrics and Gynaecology
Ullevål Hospital
Oslo
Norway

by clearance of hydrogen (9) Dilts et al. (4) found the same percentage in the non-pregnant and puerperal ewe. Calculated on the values of human uterine blood flow obtained by Jansson (6) with the Xe^{133}-clearance method the percentage would be considerably lower and Kalman (7) found uterine blood flow after oestrogen injection in rats to be about 0 2 % of cardiac output.

The electromagnetic flowmeter technique was used by Assali et al. (3) to record flow in the uterine artery in pregnant women. In 5 cases in whom hysterectomies were performed, the uteri were between 10 and 17 weeks of gestation with weights in the range of 593 and 1 020 g and blood flow in one uterine artery between about 26 and 45 ml/min. Even if absolute uterine blood flow in non-pregnant and pregnant women cannot be compared, because the latter group also includes flow to fetus and placenta it is remarkable that the flow in pregnant women was not found to be higher Possibly the reason could be that it is more difficult to expose the uterine artery in pregnant women and that the pregnant uterus and the vessels are more sensitive to the manipulation during dissection than the non pregnant uterus.

By the electromagnetic flowmeter method blood flow can be measured continuously and changes in blood flow are therefore readily obtained Utilizing this advantages, oxytocin injections were found to reduce flow in the uterine artery in all patients except one to less than 75 % of control, and in no patient did flow increase In preliminary measurements, considerable reduction of local myometrial blood flow after oxytocin injection was also observed by hydrogen clearance technique (10). This is in accordance with the observation of Assali et al (2) in unanaesthetized sheep where blood flow in the uterine artery was reduced by 25 % after intravenously administered oxytocin on the tenth day post partum.

Several factors might cause a reduction of uterine arterial blood flow depending on whether the site of action of oxytocin is the blood pressure the vessels or the myometrium. Kitchin et al. (8) observed a fall in systolic and diastolic blood pressure, and a rise in limb blood flow and cardiac output after oxytocin injection. The calculated vascular resistance before and after oxytocin in the present study showed that the decrease in blood pressure could not explain the whole reduction of flow indicating active or passive uterine vasoconstriction.

Observations on the effect of oxytocin on the uterus are controversial and complex but most investigators seem to agree that oxytocin induces uterine contractions in the presence of oestrogens. In the present study uterine blood flow decreased considerably in both the proliferative and the secretory phase.

In the pregnant ewe uterine artery flow reduction was observed with each uterine contraction varying with the intensity of the contraction recorded by palpation (1) and by simultaneous measurements of intrauterine pressure (5). No such comparison was made in the present study but the blanching of the uterine surface indicates uterine contraction although vasoconstriction cannot be excluded In patient 6 no reduction in flow was obtained after injection of oxytocin The patient was only 38 years old and had regular menstrual periods, and the observation cannot be satisfactorily explained.

The doses of Methergin the method of administration and the observation time in this study seem to be satisfactory for recording the possible effect. The lack of flow reduction suggesting no effect on either vessels or myometrium is in agreement with observations on unanaesthetized sheep (2) in which the action of Ergonovine (methergin) on flow in the uterine artery was negligible on the tenth day post partum. Even with the small number of cases, the results support the assumption that the therapeutic effect of Methergin on uterine bleeding in non-pregnant and non-puerperal women is questionable as far as blood flow is involved.

ACKNOWLEDGEMENT

I am most grateful t engineer E. Frøsteben, Institute for Experimental Medical Research, University of Oslo, Ullevål Hospital for his advice and technical help.

REFERENCES

1 Assali, N S., Dasgupta, K Kolin, A. & Holms, L. Measurement f uterine blood flow and uterine metabolism. V Changes during spontaneous and induced labor in unanaesthetized pregnant sheep and dogs. Amer J Physiol 195 614–620, 1958.
2. Assali, N S Dasgupta, K. & Kolin, A. Measurement of uterine blood flow and uterine metabolism.

Acta Obstet Gynec Scand 52 323–326, 1973

AMNIOTIC FLUID CREATININE, URIC ACID AND UREA AS INDICES OF GESTATIONAL AGE

Eng Soon Teoh, Y. K. Lau, Anselm Ambrose and S. S. Ratnam

From the Department of Obstetrics and Gynaecology (Head, Professor S. S. Ratnam) University of Singapore, Kandang Kerbau Hospital, Singapore

Abstract. Amniotic fluid creatinine, uric acid and urea were measured by an automated method in 127 pregnancies. The concentration of each solute increased with gestational age. A creatinine concentration of 2.0 mg/100 ml could be used to pick up 52.9% of mature fetuses (i.e. fetuses which had passed the 37th week of gestation) but there was a false positive pick-up of 5%. A uric acid concentration of 5.0 mg/100 ml correctly indicated a mature fetus in 41.5% of patients, the false positive pick-up being 3.3%. Amniotic fluid urea was not a useful index of fetal maturity.

When the last menstrual period is forgotten, the assessment of fetal maturity ceases to be an exact science. Since the principal cause of neonatal death is hyaline membrane disease, the exact assessment of fetal lung maturity is important in the management of all high risk pregnancies. The lecithin concentration in amniotic fluid and the lecithin sphingomyelin ratio appear to be correlated with fetal pulmonary maturity although contrary observations have now been reported (4, 5). It has been suggested that renal maturity reflected in the creatinine and uric acid concentrations in amniotic fluid, is also a useful indicator of fetal maturity (3, 7, 9).

MATERIALS AND METHOD

Amniotic fluid samples were collected from 94 patients at various stages of pregnancy and from an additional 33 patients just prior to the induction of labour. These samples were centrifuged at 3,000 r.p.m. and stored at −20°C. Before performing each assay the samples were thawed and mixed by vigorous shaking. The clear supernatant was then removed for assay. The creatinine, uric acid and urea concentrations were measured by an Auto-Analyzer system (Technicon), using Standard Auto-Analyzer Methodology

RESULTS

The mean concentrations of creatinine, uric acid and urea in amniotic fluid increased as pregnancy progressed, the slope being steepest for creatinine and least steep for urea (Fig. 1). The mean amniotic fluid creatinine concentration was 0.3 mg/100 ml at 10 weeks gestation. It increased gradually to reach 1.0 mg/100 ml at 30 weeks gestation and then rose steeply to reach 1.8 mg/100 ml at 35 weeks. At term the mean creatinine concentration was 2.0 mg/100 ml. The mean amniotic fluid uric acid concentration was 1.5 mg/100 ml at 10 weeks, doubling to 2.9 mg/100 ml at 30 weeks. It then increased rapidly to 4.0 mg/100 ml at 35 weeks and 4.9 mg/100 ml at term. There was no rapid increase in the mean urea concentration during the last trimester, urea concentrations being 12.5 mg/100 ml at 10 weeks, 21.4 mg/100 ml at 30 weeks and 27.1 mg/100 ml at term.

The scattergram of amniotic fluid creatinine in 127 patients is shown in Fig. 2 with the mean creatinine concentration at term (2.0 mg/100 ml), drawn in as a broken horizontal line. Before a gestational age of 37 completed weeks, only 2 values out of the 44 collected between 30–36 weeks fell on or above this line. Out of the 70 samples collected between 37–44 weeks, 37 or 52.9% were on or above this line. The data suggest that amniotic fluid creatinine values of 2 mg/100 ml or higher are indicative of a fetal gestational age greater than 37 weeks but in only 53% of pregnancies past 37 weeks was this level obtained. There was a false positive rate of 5% (2/39 cases).

Obstetrics and Gynecology

4 000 current abstracts in 10 fully indexed issues a year

Classification

1 GENERAL SUBJECTS
History books
Embryology
Congenital malformations
Chromosomal pattern
Heredity

2. OBSTETRICS
Normal pregnancy (mother placenta fetus)
Abnormal pregnancy (abortion trophoblastic disease ectopic toxemia and hypertension diseases complicating pregnancy blood group and rhesus antagonism hormonal aspects prolonged pregnancy fetal distress obstetric procedures)
Normal labour (physiology drugs analgesia and anesthesia fetal control)
Abnormal labor (induction dystocia and malpresentation placenta praevia solutio placentae hemorrhage obstetric operations)
Puerperium
Newborn (physiology pathology congenital malformations)
Mortality (maternal perinatal)

3. GYNECOLOGY
Endocrinology
Menstrual cycle
Reproduction (physiology sterility anticonception sexology)
Climacterium and menopause
Vulva and vagina
Uterine body and cervix
Ovaries and fallopian tubes
Gynecologic aspects of breast disease
Endometriosis
Gynecologic procedures
Cancer (etiology and incidence symptoms and detection treatment and results)
Surgery
Urology
Radiology

Annual subscription rate U S $ 68.50 / Dfl 215.00
Specimen copies will be sent on request
Quotations on back volumes will be provided on request

Excerpta Medica P O Box 211 Amsterdam, The Netherlands

order form

Please send this order form to
Excerpta Medica P O Box 211
Amsterdam The Netherlands

☐ Please enter my subscription to
OBSTETRICS AND GYNECOLOGY
Annual subscription rate U S $ 68 50 / Dfl 215 00
☐ Please send me a specimen copy
☐ Cheque enclosed ☐ Please bill me

NAME

ADDRESS

6016 EM

Acta Obstet Gynec Scand 52: 323–326, 1973

AMNIOTIC FLUID CREATININE, URIC ACID AND UREA AS INDICES OF GESTATIONAL AGE

Eng Soon Teoh, Y. K. Lau, Anselm Ambrose and S. S. Ratnam

From the Department of Obstetrics and Gynaecology (Head, Professor S. S. Ratnam) University of Singapore, Kandang Kerbau Hospital, Singapore

Abstract. Amniotic fluid creatinine, uric acid and urea were measured by an automated method in 127 pregnancies. The concentration of each solute increased with advancing age. A creatinine concentration of 2.0 mg/100 ml could be used to pick up 52.9% of mature fetuses (i.e. fetuses which had passed the 37th week of gestation) but there was a false positive pick-up of 5%. A uric acid concentration of 5.0 mg/100 ml correctly indicated a mature fetus in 41.5% of patients, the false positive pick-up being 3.3%. Amniotic fluid urea was not a useful index of fetal maturity.

When the last menstrual period is forgotten, the assessment of fetal maturity ceases to be an exact science. Since the principal cause of neonatal death is hyaline membrane disease, the exact assessment of fetal lung maturity is important in the management of all high risk pregnancies. The lecithin concentration in amniotic fluid and the lecithin sphingomyelin ratio appear to be correlated with fetal pulmonary maturity although contrary observations have now been reported (4, 5). It has been suggested that renal maturity reflected in the creatinine and uric acid concentrations in amniotic fluid is also a useful indicator of fetal maturity (3, 7, 9).

MATERIALS AND METHOD

Liquor samples were collected from 94 patients at various stages of pregnancy and from an additional 33 patients just prior to the induction of labour. These samples were centrifuged at 2000 r.p.m. and stored at –20°C. Before starting each assay the samples were thawed and mixed by vigorous shaking. The clear supernatant was then removed for assay. The creatinine, uric acid and urea concentrations were measured by an Auto-Analyser system (Technicon), using Standard Auto-Analyser Methodology.

RESULTS

The mean concentrations of creatinine, uric acid and urea in amniotic fluid increased as pregnancy progressed, the slope being steepest for creatinine and least steep for urea (Fig. 1). The mean amniotic fluid creatinine concentration was 0.3 mg/100 ml at 10 weeks gestation. It increased gradually to reach 1.0 mg/100 ml at 30 weeks gestation and then rose steeply to reach 1.8 mg/100 ml at 35 weeks. At term the mean creatinine concentration was 2.0 mg/100 ml. The mean amniotic fluid uric acid concentration was 1.5 mg/100 ml at 10 weeks, doubling to 2.9 mg/100 ml at 30 weeks. It then increased rapidly to 4.0 mg/100 ml at 35 weeks and 4.9 mg/100 at term. There was no rapid increase in the mean urea concentration during the last trimester, urea concentrations being 12.5 mg/100 ml at 10 weeks, 21.4 mg/100 ml at 30 weeks and 7.1 mg/100 ml at term.

The scattergram of amniotic fluid creatinine in 177 patients is shown in Fig. with the mean creatinine concentration at term (2.0 mg/100 ml) drawn in as a broken horizontal line. Before a gestational age of 37 completed weeks, only 2 values out of the 4 collected between 30–36 weeks fell on or above this line. Out of the 70 samples collected between 37–44 weeks, 37 or 52.9% were on or above this line. The data suggest that amniotic fluid creatinine values of mg/100 ml or higher are indicative of a fetal gestational age greater than 37 weeks but in only 53% of pregnancies past 37 weeks was this level obtained. There was a false positive rate of 5% (? 39 cases).

Obstetrics and Gynecology

4 000 current abstracts in 10 fully indexed issues a year

Classification

1 GENERAL SUBJECTS
   History books
   Embryology
   Congenital malformations
   Chromosomal pattern
   Heredity

2. OBSTETRICS
   Normal pregnancy (mother placenta fetus)
   Abnormal pregnancy (abortion trophoblastic disease ectopic toxemia and hypertension diseases complicating pregnancy blood group and rheaus antagonism hormonal aspects prolonged pregnancy fetal distress obstetric procedures)
   Normal labour (physiology drugs analgesia and anesthesia fetal control)
   Abnormal labor (induction dystocia and malpresentation placenta praevia solutio placentae hemorrhage obstetric operations)
   Puerperium
   Newborn (physiology pathology congenital malformations)
   Mortality (maternal perinatal)

3. GYNECOLOGY
   Endocrinology
   Menstrual cycle
   Reproduction (physiology sterility anticonception sexology)
   Climacterium and menopause
   Vulva and vagina
   Uterine body and cervix
   Ovaries and fallopian tubes
   Gynecologic aspects of breast disease
   Endometriosis
   Gynecologic procedures
   Cancer (etiology and incidence symptoms and detection treatment end results)
   Surgery
   Urology
   Radiology

Annual subscription rate U.S $ 68.50 / Dfl 215 00
Specimen copies will be sent on request
Quotations on back volumes will be provided on request

Excerpta Medica P O Box 211 Amsterdam, The Netherlands

order form

Please send this order form to Excerpta Medica P O Box 211 Amsterdam The Netherlands

☐ Please enter my subscription to OBSTETRICS AND GYNECOLOGY
   Annual subscription rate U S $ 68.50 / Dfl 215 00
☐ Please send me a specimen copy
☐ Cheque enclosed ☐ Please bill me

NAME

ADDRESS

6016 EM

The scattergram of amniotic fluid uric acid is shown in Fig. 3 and a horizontal line is drawn across 5 mg/100 ml, 0.1 mg above the mean uric acid concentration at term. Before 37 weeks gestation only one sample showed a concentration above 5 mg/100 ml. Of the 70 samples collected from the 37th week onward, 29 had uric acid concentrations of 5 mg/100 ml or were higher. Thus, if 5 mg/100 ml is used as a demarcation line 41.5% of mature fetuses would be recognized by this determination and there was a false positive of 3.3% (1/30 cases).

The scattergram of amniotic fluid urea is shown in Fig. 4. While the urea concentration gradually increased with gestational age there was no definite demarcation between mature and immature fetuses.

COMMENTS

There is a common agreement that the mean amniotic fluid creatinine concentration at term is [illegible]0 mg/100 ml and a level [illegible]t this point or higher is indicative of fetal maturity (1 ... 3). Pitkin & Zwick (7) claimed that they were able to predict fetal maturity in 94% of cases, and Donnai et al. (2) obtained a 93.4% pick-up rate by lowering the critical level to 1.7 mg/100 ml. Since the measurement of creatinine and uric acid involves simple procedures which are routine in most service laboratories this should be an easy method of estimating fetal maturity. However, our present study indicates that only 50% of mature fetuses would be recognized through amniotic fluid creatinine and there is an over-estimation of fetuses which have reached the 38th week in [illegible] of cases. The number of false estimates of mature fetuses caused through lowering the demarcation line was not stated by previous authors but is evident from the scattergrams in these reports ([illegible]). Donnai et al. ( ) observed that the estimation of gestational age by this method may occasionally be out by as much as four weeks.

The range of amniotic fluid uric acid concentration in mature pregnancies in the present series ([illegible] 10.0 mg/100 ml) is lower than had been previously reported (4.5–15 mg/100 ml). The present mean uric acid of 4.9 mg/100 ml is *half of* the mean reported from Western centres (9.9 mg/100 ml) (6, 9). A difference in birth weight of Singapore and American babies is a possible explanation. However birth weight did not correlate with uric acid concentration in the present series. Harrison (6) claimed a detection rate for mature fetuses of 79% using amniotic fluid uric acid. Using a different demarcation level for uric acid of 5 mg/100 ml, since our scatter was different, we were only able to recognize 41.5% of the mature fetuses. There were insufficient cases in Wolf's series to indicate the percentage of false positives but the possibility of this is evident from the shape of the scattergram and from our own observation of false positives in 3.3% of cases.

It is important to select an appropriate demarcation line in order to ensure that premature infants are not delivered. As the critical level is elevated the risk of false positives decreases but so does the pick-up rate. Donnai et al. (2) showed, that when amniotic fluid creatinine was combined with Nile Blue Sulphate staining, a higher pick-up rate could be achieved. At the present time it appears that a thorough exploitation of amniotic fluid analyses for fetal maturity should include *Nile Blue Sulphate* staining, creatinine concentration, uric acid concentration, lecithin concentration and the lecithin/sphingomyelin ratio.

ACKNOWLEDGEMENT

The authors are grateful to Dr Paul Tan and Dr A. K. Tay for assistance in collecting some of the liquor specimens and to the Department of Biochemistry, Outram Road General Hospital, Singapore, for the use of the Auto-Analyzer.

REFERENCES

1. Benitrez, G. C., Perkins, P. & Wasmus, B.: Amer J Obstet Gynec *106*: 917, 1970.
2. Donnai, P., Gordon, H., Harris, D. A. & Hopkin, E. A.: J Obstet Gynaec Brit Comm *78*: 603, 1971.
3. Doran, T. A., Bjerre, S. & Porter, C. J.: Amer J Obstet Gynec *106*: 325, 1970.
4. Gluck, L., Kulovich, M. V., Borer, R. C., Brenner, P. H., Anderson, G. G. & Spellacy, W. N.: Amer J Obstet Gynec *109*: 440, 1971.
   Gordon, J. P. & Moseley Ware, B.: Amer J Obstet Gynec *11*[illegible] 6[illegible], 1972.
6. Harrison, R. F.: J Obstet Gynaec Brit Comm *79*: 70[illegible], 1972.
7. Pitkin, R. M. & Zwirek, S. J.: Amer J Obstet Gynec *9*[illegible]: 1[illegible], 1967.

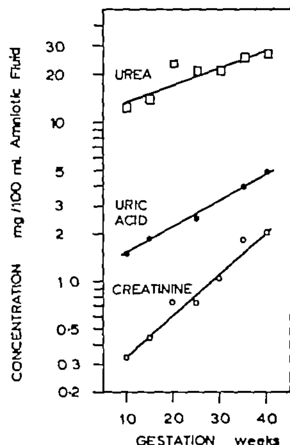


Fig 1 Mean amniotic fluid concentrations of creatinine uric acid and urea in 127 Asian subjects.

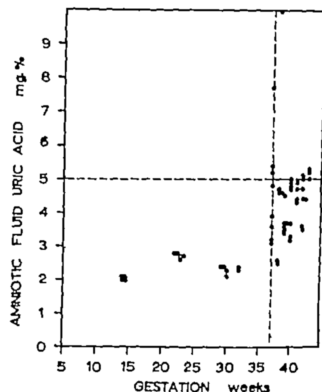


Fig 3 Amniotic fluid uric acid. A level of 5 mg/100 ml or higher is indicative of a mature fetus.

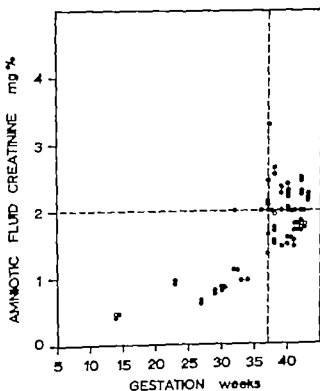


Fig 2 Amniotic fluid creatinine A level of 2 mg/100 ml or higher is indicative of a "mature" fetus (i.e. past 37 weeks).

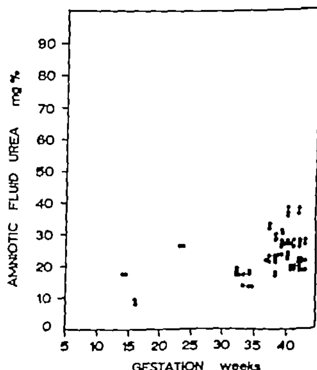


Fig 4 Amniotic fluid urea. Because of the wide scatter there is no reliable demarcation line for the mature fetus.

The scattergram of amniotic fluid uric acid is shown in Fig. 3 and a horizontal line is drawn across 5 mg/100 ml, 0.1 mg above the mean uric acid concentration at term. Before 37 weeks gestation only one sample showed a concentration above 5 mg/100 ml. Of the 70 samples collected from the 37th week onward, 29 had uric acid concentrations of 5 mg/100 ml or were higher. Thus, if 5 mg/100 ml is used as a demarcation line 41.5% of mature fetuses would be recognized by this determination, and there was a false positive of 3.3% (1/30 cases).

The scattergram of amniotic fluid urea is shown in Fig. 4. While the urea concentration gradually increased with gestational age, there was no definite demarcation between mature and immature fetuses.

COMMENTS

There is a common agreement that the mean amniotic fluid creatinine concentration at term is 2.0 mg 100 ml and a level at this point or higher is indicat e of fetal maturity (1 2, 3). Pitman & Z rek (7) claimed that they were able to predict fetal maturity in 94% of cases, and Doonai et al ( ) obtained a 93.4% pick-up rate by lowering the critical level to 1.7 mg/100 ml. Since the measurement of creatinine and uric acid in ol ves simple procedures which are routine in most servic laboratories this should be an easy method of estimating fetal maturity. However our present study indicates that only 50% of mature fetuses would be recognized through amniotic fluid reatinine and there is an over-estimation of fetuses which ha e reached the 38th week in (of ases. The number of false estimates of mature f tuses caused through lowering the demarc ti n line as not stated by previous authors but was evident from the scattergrams in these report ( ). Doonai et al ( ) observed that the estimation of gestational age by this method m sionally be out by as much a four eek.

The ange of mniotic fluid uric acid concentr tion in mature pregnancies in the present series (2 10.0 mg 100 ml) is lower than had been prev ously reported (4.5–15 mg 100 ml). The present mean uric acid of 4.9 mg 100 ml is half of the me n report d from Western centres (9.9 mg 100 ml) (6 9). A difference in birth weight of Singapore and American babies is a possible explanation. However birth weight did not correlate with uric acid concentration in the present series. Harrison (6) claimed a detection rate for mature fetuses of 79% using amniotic fluid uric acid. Using a different demarcation level for uric acid of 5 mg/100 ml since our scatter was different, we were only able to recognize 41.5% of the mature fetuses. There were insufficient cases in Wolf's series to indicate the percentage of false positives but the possibility of this is evident from the shape of the scattergram and from our own observation of false positives in 3.3% of cases.

It is important to select an appropriate demarcation line in order to ensure that premature infants are not delivered. As the critical level is elevated the risk of false positives decreases but so does the pick-up rate. Doonai et al. (2) showed, that when amniotic fluid creatinine was combined with Nile Blue Sulphate staining, a higher pick up rate could be achieved. At the present time it appears that a thorough exploitation of amniotic fluid analyses for fetal maturity should include Nile Blue Sulphate staining, creatinine concentration, uric acid concentration, lecithin concentration and the lecithin/sphingomyelin ratio.

ACKNOWLEDGEMENT

The authors are grateful to Dr Paul Tan and Dr A. K. T y for assistance in collecting some of the liquor specimens and to the Department of Biochemistry, Outram Road General Hospital, Singapore for the use of the Auto-Analyzer.

REFERENCES

1 Benfrero, G. C. Pedues, P & W uuan, B. Amer J Obstet Gynec 106 917 1970
2 Doonai, P Gordon, H Harris, D A & Hughes, E. A J Obstet Gynaec Brit Comm 78 603 1971
3 Doran, T A., Byrne S & Perler C J Amer J Obstet Gynec 106 325 1970
4 Gluck, L., Kulovich, M V Borer R. C Brenner P H Anderson, G G & Spellacy W N Amer J Obstet Gynec 109 440, 1971
Gordon, J P & Moseley Wade, R Amer J Obstet Gynec 11 6 1972
6. Harrison, R. F J Obstet Gynaec Brit Comm 79 708 1972
7 Pitkin, R. M & Zwirek, S. J Amer J Obstet Gynec 98 333 1967

8. Technicon Auto-Analyser Methodology Technicon Corporation, Tarrytown, New York, 1967
9 Wolf, P L, Bloch, D & Tsudaka, T.: Clin Chem *16* 843 1970.

Submitted for publication Nov 8 1972

Eng Soon Teoh
Department of Obstetrics and Gynaecology
University of Singapore
Kandang Kerbau Hospital
Singapore 8

Acta Obstet Gynec Scand 52, 327–334 1973

^{133}XENON CLEARANCE FROM THE HUMAN NON PREGNANT UTERUS

Comparison between Elimination Curves after Local and Intra-arterial Injection of Tracer

Lars Forssman

From the Department of Obstetrics and Gynecology (Head: Professor Per Bergman), Östra sjukhuset Göteborg, Sweden

Abstract. A study of the clearance of ^{133}Xenon from the non pregnant human uterus is reported. The isotope was deposited in the uterus either by direct injection at fixed depth from the endometrial surface in the uterine fundus or by injection into the uterine artery. With both types of injection multiexponential curves resulted. This is thought to reflect the existence within the uterus of compartments with different rates of perfusion. By conventional graphical separation of curve components two, sometimes three, components are identified. Their mean half times after intraarterial injection in 15 cases 1.4 and 11.4 min respectively. After local injection in 10 cases 1.2 and 20 min respectively. A third very quick component was seen in carcinoma cases and is not calculated.

In animal experiment uterine blood flow can be studied by a variety of methods (4, 5, 23). Many of these involve major surgery and in some methods the animal has to be sacrificed. Work in the field of human uterine blood flow therefore has been directed mainly towards the finding of a suitable method that would allow repeated studies in the same individual under different conditions. The principles mainly applied are the following: heat-clearance (22), Hydrogen-clearance (10, 11), isotope-clearance according to Kety—for review see Jansson (7).

In a previous report (21) a new method was described to study the uterine blood flow after local injection of ^{133}Xe. This method was applied to a series of cases with carcinoma of the uterine body. Significant differences were found depending on the histological differentiation of the tumour.

For further evaluation of the method a group of healthy volunteers was studied. The same technique of isotope injection was used but more sophisticated recording equipment made a detailed analysis of the elimination curve possible. It was then found that the elimination of ^{133}Xe did not follow a straight line when plotted in a semilogarithmic system. The slope of the desaturation curve was greatest in the beginning decreasing towards the end of the recording time. The explanation of this was not clear. The effect of anaesthesia could be excluded since some measurements were made with, others without general anaesthesia resulting in the same type of curve. Leakage of isotope via the needle tract could not be excluded as the cause of the initially shorter half-times. Neither could reactive hyperaemia due to tissue trauma during injection be excluded. These two explanations, however seemed less probable since local injection into the myometrium near the serosa during laparotomy had not given the same fast initial component (Forssman unpublished data).

Injection of isotope in the artery supplying the region to be studied gives the possibility of depositing the isotope without mechanical interference with the tissue. If the injected artery supplies several regions with different rates of blood flow each compartment is labelled in proportion to its blood flow provided the partition coefficients are the same. Consequently the elimination curve reflects blood flow in all labelled regions. The risk inherent in the local injection technique of labelling a small non-representative volume of tissue is eliminated if the main artery of the organ is used for the injection. The intra-arterial labelling technique reduces the risk of isotope leakage to the risk of diffusion from the organ surface and

8. Technicon Auto-Analyser Methodology Technicon Corporation, Tarrytown, New York, 1967
9 Wolf, P L, Bloch, D & Tsudaka, T Clin Chem *16* 843 1970.

Submitted for publication Nov 8 1972

Eng Soon Teoh
Department of Obstetrics and Gynaecology
University of Singapore
Kandang Kerbau Hospital
Singapore 8

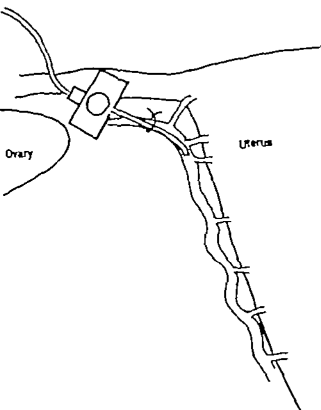


Fig. 1. Schematic drawing showing the Venflon catheter introduced via the right ovarian branch into the uterine artery

the needle tract the needle was left in situ for 20 sec after the injection before withdrawal. For these studies no premedication or anaesthesia was used except in the two cases that were studied prior to laparotomy.

3. *Detecting and recording system*

A lead collimated scintillation detector was connected to a pulse height analyzer adjusted to the 81 KeV gamma radiation peak of ^{133}Xe. A ratemeter was connected to a linear writing recorder (equipment: AB Nukab, Goteborg). At laparotomy the detector with a sterile covering was placed with the lower end of the collimator 1 cm from the uterine surface, the collimator angle allowing the uterus and structures immediately behind to be seen by the detector. When the injection was made via the right ovarian branch of the uterine artery a 5 mm lead shield was used to shield the catheter system from the detector.

When local injections were made the detector was placed above the symphysis pubis and adjusted to cover the pelvic viscera. The recording time was between 15 and 90 minutes. At the beginning of the recording the time constant of the ratemeter was 1 sec then step-wise increasing to 3 and 10 sec. The range of the ratemeter was changed step-wise from 100 000 counts per minute to 3 000 counts per minute. Paper speed was kept constant at 4 m min. Back-ground activity was checked before and after each measurement.

4. *Curve analysis*

The logarithm of the linearly recorded activity minus back-ground was plotted on semilogarithmic paper against time. The tail portion of each curve was approximated to a straight line and the values of the extrapolated tail portion were subtracted from the original curve. The values obtained formed a new curve on which the procedure could be repeated if necessary. From the curves obtained the halftimes were calculated.

RESULTS

In all cases studied the curves recorded were of the multiexponential type. In this respect there were no differences between intra-arterial and local injections in the special cannula. Conventional graphical analysis of the curves yielded the results presented in Tables I and II. Typical curves after intra-arterial and after local injections are shown in Figs. 2 and 3. In one case the catheter was inadequately shielded which makes determination of the slope of the tail portion of the curve impossible. As can be judged by the initial portion of the curve it is of the same type as the others. Since the case was not correctly recorded

the risk of vasomotor nerve reflexes elicited by the manipulation of the vessel may be reduced by the use of spasmolytics and local anaesthetics.

The present investigation, which for the first time uses intra-arterial isotope injection to the human uterus, was planned to answer the following questions:

1. Is the elimination curve in a semilogarithmic system after intra-arterial injection of ^{133}Xenon to the uterus a straight or a curved line?

2. If not a straight line—may the elimination curve be broken down into components with different slopes?

3. Does there exist any resemblance between curves after local and intra-arterial injections of tracer?

METHODS AND MATERIAL

1. *Theoretical considerations*

A rapidly diffusing, inert indicator is cleared from a constantly and uniformly perfused tissue according to equation (1).

$$C_t = C_0 \cdot e^{-kt} \qquad (1)$$

where C_0 and C_t stand for amount of indicator at time 0 and time t.

k is the clearance constant and may be derived from equation (2)

$$k = \frac{\ln 2}{t\frac{1}{2}} \qquad (2)$$

$t\frac{1}{2}$ is the half-time for the elimination of the indicator.

Equation (1) gives a straight line when plotted in a semilog. system. If the tracer leaves the tissue only by the blood stream, if recirculation of indicator and concentration gradients within the tissues may be neglected, blood flow can be calculated by equation (3)

$$Q = k \cdot \lambda \cdot 100 \qquad (3)$$

where Q is blood flow expressed as ml blood min^{-1} 100 g^{-1}, λ is the tissue/blood partition coefficient expressed as ml blood/g tissue.

Equation (1) is valid only if the tissue is uniformly perfused. If there exist compartments within the labelled region with different rates of perfusion the situation may be described by equation (4)

$$C_t = C_{0_1} \cdot e^{-k_1 t} + C_{0_2} \cdot e^{-k_2 t} + C_{0_3} \cdot e^{-k_3 t} + \ldots + C_{0_n} \cdot e^{-k_n t} \qquad (4)$$

where C_{0_1}, C_{0_2} ... C_{0_n} stand for the amount of indicator in each compartment at time zero. k_1, k_2 ... k_n are the different clearance constants. If this equation is plotted in a semilog. system a curved line results. This multi-exponential function may be analysed into its components by repeated exponential subtractions. The local clearance method of measuring blood flow was originally described by Kety (18) using ^{24}Na. Since Na is not freely diffusible but passes the capillary wall via water filled pores ^{24}Na gives correct values for regional blood-flow only at low perfusion rates. At higher rates the PS-product, i.e. the product between perfused area of capillary surface (S) and permeability (P) for the tracer is the limiting factor.

^{133}Xe and ^{85}Kr both being lipophilic use the whole of the capillary wall for their passage. The blood–tissue permeability is great compared with blood flow and the clearance is thus flow limited (3, 1.., 13).

2. *Material and operative procedure*

Patients to be subjected to total hysterectomy for gynecological disease were chosen for the study of intra-arterial injections provided the size of the uterus did not exceed the size of a uterus 12 weeks pregnant. Fifteen patients were studied. The cases are presented together with results in Table I. General anaesthesia with muscle relaxation was used. Ventilation was assisted, no spontaneous respiration allowed. To prevent re-breathing of isotope a semi-open non-rebreathing system was used. Blood pressure was in all cases within normal levels, showing only minor variations during the measurements. The patients were placed in the supine position on the operating table, the head end lowered 5 to 10 degrees. In the first 5 cases the right uterine artery was exposed and dissected free from the surrounding tissue for a distance of 1–2 cm immediately lateral to its crossing the right ureter. The vessel was soaked with 1% lidocaine (Xylocain® Astra) to reduce the risk of vasospasm during the dissection and injection. The artery was punctured with a thin cannula (outer diameter 0.4 mm) and 50–100 μCi of ^{133}Xe in 0.1–0.2 ml of 0.9% NaCl-solution (AB Atomenergi, Studsvik) was injected quickly (1 sec). Thereafter the cannula was withdrawn. As a rule there was some leakage through the puncture hole. This blood was immediately removed by suction.

In the later cases the right uterine artery was cannulated via its right ovarian branch with a no. 100 Venflon catheter (AB Viggo, Helsingborg) connected to a polyethylene catheter (Fig. 1). The system was filled with heparin solution to prevent clotting (2 500 IU Heparin/100 ml of 0.9% NaCl solution). The isotope injections were carried out in the retrograde direction into the right uterine artery. The injected amount of isotope was the same as in the antegrade injections.

For comparison 2 groups were given injections by the transuterine technique previously described (21). There were 6 cases of cancer of the uterine body and 4 young menstruating women with no known gynecological disease. Also two of the intraarterially injected cases were studied by the local injection technique immediately prior to the intra-arterial study. The cases are presented together with results in Table II. A thin flexible cannula with a bulb-shaped tip was passed through the cervical canal and uterine cavity until it reached the uterine fundus where it was held in contact with the uterine wall. Via this outer cannula an inner cannula—external diameter 0.4 mm—was passed until its end protruded 7 mm from the outer cannula. Via the inner cannula an injection of 0.1–0.2 ml of isotope solution (50–100 μCi) was given. The duration of the injection was 30 sec. To prevent injection of gas bubbles no aspiration was made prior to injection. To reduce the risk of reflux along

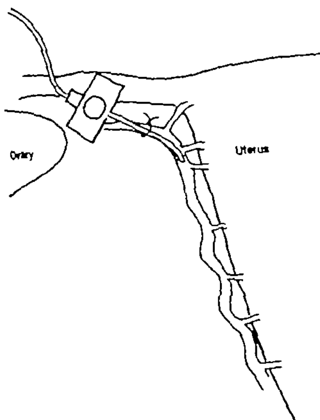


Fig. 1. Schematic drawing showing the Venflon catheter introduced via the right ovarian branch into the uterine artery.

he needle tract the needle was left in situ for 20 sec after the injection before withdrawal. For these studies no premedication or anaesthesia was used except in the two cases that were studied prior to laparotomy.

3. *Detecting and recording system*

A lead collimated scintillation detector was connected to a pulse height analyzer adjusted to the 81 KeV gamma radiation peak of ^{133}Xe. A ratemeter was connected to a recti-rating recorder (equipment AB Nukab, Göteborg). At laparotomy the detector with sterile covering was placed with the lower end of the collimator 1.5 cm from the uterine surface, the collimator angle allowing the uterus and structures immediately behind it to be seen by the detector. When the injection was made via the right ovarian branch of the uterine artery 5 mm lead shield was used to shield the catheter system from the detector.

When local injections were made the detector was placed above the symphysis pubis and adjusted to cover the pelvic viscera. The recording time was between 1 and 30 minutes. At the beginning of the recording the time constant of the ratemeter was 1 sec then step-wise increasing to 3 and 10 sec. The range of the ratemeter was changed step-wise from 100 000 counts per minute to 3 000 counts per minute. Paper speed was kept constant at 4 in./min. Back-ground activity was checked before and after each measurement.

4. *Curve analysis*

The logarithm of the linearly recorded activity minus back-ground was plotted on semilogarithmic paper against time. The tail portion of each curve was approximated to a straight line and the values of the extrapolated tail portion were subtracted from the original curve. The values obtained formed a new curve on which the procedure could be repeated if necessary. From the curves obtained the halftimes were calculated.

RESULTS

In all cases studied the curves recorded were of the multiexponential type. In this respect there were no differences between intra-arterial and local injections via the special cannula. Conventional graphical analysis of the curves yielded the results presented in Tables I and II. Typical curves after intra-arterial and after local injections are shown in Figs. 2 and 3. In one case the catheter was inadequately shielded which makes determination of the slope of the tail portion of the curve impossible. As can be judged by the initial portion of the curve it is of the same type as the others. Since the case was not correctly recorded

Table I *Cases injected intra-arterially*

The 5 first cases injected into the uterine artery at the ureter crossing. The rest via catheter in the ovarian branch

				First component		Second component	
Patient	Age (y)	Diagnosis	Cycle phase of endometrium	$t\frac{1}{2}$ min	Q ml min^{-1} 100 g^{-1}	$t\frac{1}{2}$	Q ml min^{-1} 100 g^{-1}
B B	37	Myoma	Proliferative	1.5	32.3	7.5	6.6
E. J	48	Endometrial Ca. st. 0[a]	Proliferative	1 7	28.5	37	1.3
U J	47	Endometrial Ca. st. 0[a]	Proliferative	1.8	26.9	18.5	2.6
S. H	46	Adenomyosis	Proliferative	1.5	32.3	11.3	4.2
R. J	35	Myoma	Secretory	1.2	40.4	4 8	10.1
B. H	51	Myoma	Proliferative	3.5	13.9	8	6.1
K. W	28	Endometrit. chron.	Secretory (?)	1.0	48.5	11.5	4.2
I T	54	Endometrial Ca. st. 0[a]	Proliferative	1.3	37 3	9.5	5.1
L. P	37	Myoma	Oral contraceptives[b]	1 4	34 7	10	4.8
H O	38	Endometrial Ca st. 0[a]	Secretory	1 0	48.5	19	2.5
K A.	41	Myoma	Proliferative	1 1	44 1	4 8	10 1
M S.	46	Myoma	Secretory	1 6	30.3	11 6	4.2
K. D	50	Myoma	Secretory	1 4	34 7	1	4 0
I J	33	Dysmenorrhoea	Secretory	1.2	40.4	6.3	7 7

Endometrial Ca. st. 0 stands for premalignant typical proliferation of glandular epithelium in the endometrium. In some cases the atypical proliferation was confined to a small polyp, in others a more wide-spread atypia was seen.
[b] Combined type. Discontinued a few days before operation.
Short recording time 9.5 min and 14 min respectively

it is excluded from the tables and calculations. In calculations of blood flow figures a partition coefficient of 0 7 has been assumed—this was calculated for skeletal muscle (1)

After intra-arterial injections the following values were calculated. First component. mean half time 1 4 min. Range 1 0–3.5 min corresponding to a mean blood flow of 34 7 ml min^{-1} 100

Table II *Cases injected via the special cannula*

				First component		Second component	
Patient	Age (y)	Diagnosis	Cycle phase (if known)	$t\frac{1}{2}$	Q ml min 100 g	$t\frac{1}{2}$	Q ml min 100 g^{-}
S. B	61	Endometrial carcinoma	Post-menopausal	0 9	53 9	35	1 4
K. J	45	Endometrial carcinoma	—	1.5	3 3	10 2	4 8
M L.	58	Endometrial carcinoma	Post menopausal	1 3	37 3	20	2 4
M B.	63	Endometrial carcinoma	Post-menopausal	1 1	44 1	19.5	2 5
H G	54	Endometrial carcinoma	Post-menopausal	1 2	40 4	36	1 3
H. J	46	Endometrial carcinoma	—	1 1	44 1	6.3	7 7
B. S.	31	Normal	Proliferation	1.3	37 3	13 8	3 5
B. K.	21	Normal	Proliferation	1 4	34 7	18	7
L. W	24	Normal	Proliferation	1 1	44.1	30	1
B. G	24	Normal	Oral contraceptives	0.8	60 6	14	3.5
K. W	28	Same case as KW in intra-arterial series		0.9	53 9	6.5	7.5
L. P	37	Same case as LP in intra-arterial series		1.3	37 3	9.5	5 1

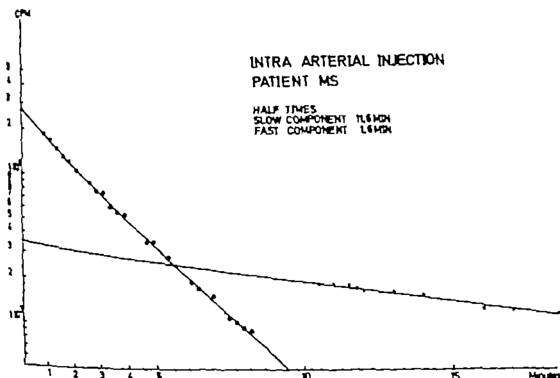


Fig. 2. Typical curve after intraarterial injection. Original curve, crosses. Curve obtained after subtraction of slow component: circles.

Second component, mean half-time 11.4 min. Range 4.8–37 min corresponding to a mean blood-flow of 4.3 ml min^{-1} 100 g. After direct injections the following values were calculated. First component: mean half-time 1.2 min. Range 0.8–1.5 min calculated mean blood-flow 40.4 ml min^{-1} 100 g. Second component, mean half-time 20.0 min. Range 6.3–50 min calculated mean blood-flow 2.4 ml min^{-1} 100 g.

In the two cases where recording after both local and intra-arterial injection was made there is good agreement between the calculated half times. The two curves from one of these cases are shown in Fig. 4.

In some cases a third, very quick component could be identified in the first minute of recording. These cases were all carcinoma cases. No attempt was made to calculate the slope of this part of the curve for reasons discussed below.

DISCUSSION

Studies of ^{133}Xe elimination after intra-arterial injection to the human uterus have not been reported earlier. This technique for isotope injection labels all regions supplied by the injected vessel. There is no risk of leakage through a needle tract as with the local injection technique, neither is there any tissue trauma by the injection needle. Thus these two possible causes of artefacts may be excluded as the origin of the observed non-linearity of the semilog. elimination curves. Leakage through diffusion from the uterine surface is less probable as the cause of the initially quicker elimination because of the constant slope of the first component of the curve. This is also verified on the cat gastrocnemius preparation by Sejrsen & Tønnesen (24).

Thus it seems reasonable to regard the elimination curves as reflecting uterine blood flow.

A similar type of elimination curve was obtained after intra-arterial administration of tracer to the brain where white and grey matter were found to have significantly different perfusion (6). The small intestine of the cat was shown to give four different components in the wash-out curve. Three of these were shown to correspond to dif

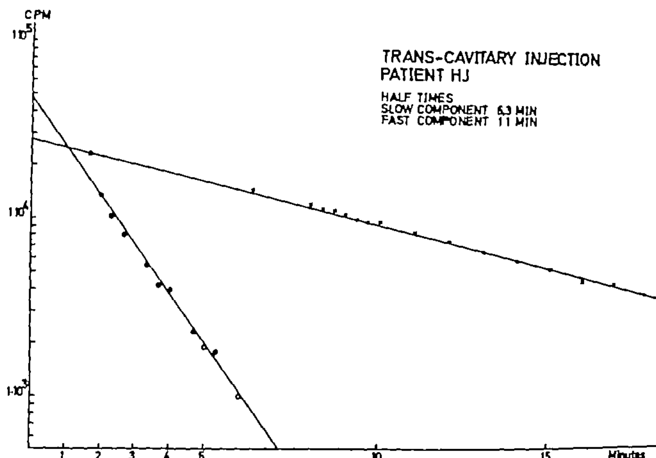


Fig. 3. Typical curve after local injection. Case of endometrial carcinoma. Note initial very quick component. Original curve: crosses. Curve obtained after subtraction of slow component: circles.

ferent anatomical structures with different perfusion. The remaining component was considered to reflect counter-current flow in the intestinal villi (18). The desaturation curve for ^{133}Xe from skeletal muscle shows a very slow tail portion. This has been thought to depend on deposition of isotope in connective tissue strands with lower perfusion than the surrounding muscle (14).

The origin of the components of the observed wash-out curves from the uterus is not as yet clear. It is possible that the recorded half times cannot be correlated to specific anatomical structures. The wash-out curve may reflect a continuum of slightly different half times in which case the constructed half times have no physiological meaning, i.e. they cannot be correlated to any specific anatomical structure (9, 17). However, through the intraarterial injection all components of the uterine wall are labelled with isotope and it therefore would seem probable that the calculated half times could be shown to correspond to different anatomical structures in the uterine wall. The endometrium and the myometrium represent the components, which are primarily suspected to be the origin of the two calculated half times.

The local transcavitary injection technique means that the depot of tracer will be located very near the borderline region between endometrium and myometrium (Fig. 5). Thus the possibility exists that both the wash-out curve after local injection and that after intra-arterial injection reflect clearance of isotope from both endometrium and myometrium.

This would account for the agreement between values calculated after local and after intra-arterial injections. However, other explanations to the agreement between the two types of curves are possible even if we still assume that the calculated values are physiologically meaningful. Connective tissue in the uterine wall probably has a slow clearance and may be the origin of the slow component. The fast component then might be a mean of two closely related components that are

TRANS-CAVITARY AND INTRA-ARTERIAL INJECTIONS
PATIENT LP

HALF TIMES

	TRANS-CAVITARY INJ.	INTRA ARTERIAL INJ.
SLOW COMPONENT	11.8 Min.	11.7 Min.
FAST COMPONENT	1.4 Min.	1.5 Min.

CPM; 10^3; 10^2; 10^1; 1 2 3 4 5 10 15 Minutes

Fig. 4. Curves obtained after local and intra-arterial injections in the same patient. Original curves: crosses. Curves obtained after subtraction of slow component: circles. Solid lines: intra-arterial injection. Interrupted lines: local injection.

not separated by this type of evaluation of the curves, representing myometrium and endometrium.

Jansson (7) found a mean myometrial blood flow of 23.0 ± 2.98 ml min^{-1} 100 g^{-1} after local injection at laparotomy in non-pregnant uteri.

Munck et al. (20) report mean values of 11 ml min^{-1} 100 g^{-1} in the cervix and 23 ml min^{-1} 100 g^{-1} in the isthmus. During pregnancy however values between 7 and 10 ml min^{-1} 100 g^{-1} have been found in myometrium remote from the placental site (2, 7).

Values have been given for endometrial blood flow in the pregnant sheep (19) and pregnant rhesus monkey (15) but expressed only as percentages of total uterine blood flow and cardiac output respectively. There is no report giving values for human endometrial blood flow. Applying the termal conductivity principle and using the Hensel needle Prill & Götz (22) found that

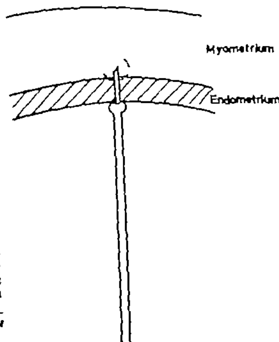


Fig. 5. Schematic drawing showing the localization of the local trans-cavitary injection.

human myometrial blood flow was significantly higher than that of skeletal muscle and that endometrial blood flow was lower than that of myometrium. Their method however did not give absolute blood flow values.

The very fast component observed in some cases was seen almost exclusively after local injection where it may be caused by removal after injection of the syringe containing isotope from the vicinity of the detector However this needs further study especially since these cases were carcinoma cases.

The questions raised in the beginning of this paper may be answered as follows.

1 The elimination of ^{133}Xenon after intra-arterial injection into the uterus describes a curved line when plotted in a semilog. system

2. The elimination curve may be broken down into at least two components with different half times.

3 Local transcavitary injection of isotope gives the same type of curve with two components with half-times very similar to those of the intra-arterial curve.

It is not possible to draw any conclusions as to the physiological meaning of the calculated half times. Further work is needed to find the anatomical counterparts of the different parts of the elimination curve.

REFERENCES

1 Conn, H. L. Equilibrium distribution of radio-Xenon in tissue: Xenon-haemoglobin association curve. J Appl Physiol 16 1065 1961
2. Falk, V Forkman, B & Lindell, S E. Xenon 133 clearance as a measure of blood flow through the human myometrium. Strahlentherapie (Sonderb.) 65 16., 1967
3. Folkow B. & Neil, E. Circulation. Oxford University Press, London and Toronto, 1971
4 Greiss, F C. A clinical concept f uterine blood flow during pregnancy Obstet Gynec 30 595 1967
5 Huckabee, W E. Uterine blood flow Amer J Obstet Gynec 84 1623 196..
6. Haggendal, E., Nilsson, N J Norbäck, B On the components of Kr^{85} clearance curves from the brain of the dog. Acta Physiol Scand 66 Suppl. 258 p 5 1965
7 Jansson, I Xenon clearance in the myometrium of pregnant and non-pregnant women. Acta Obstet Gynec Scand 48 302, 1969
8. Kety S. S. Measurement of regional circulation by the local clearance of radioactive sodium. Amer Heart J 38 321 1949
9 Kjellmer I., Lindbjerg, I Prerovsky I. & Tønnesen, H. The relation between blood flow in an isolated muscle measured with the Xe^{133} clearance and a direct recording technique. Acta Physiol Scand 69 69 1967
10. Klingenberg, I. & Aukland, K. Measurement of human uterine cervical blood flow by local hydrogen gas clearance. Acta Obstet Gynec Scand 45 455 1969
11 Klingenberg, I. Measurement of blood flow in human myometrium by local hydrogen clearance Acta Obstet Gynec Scand 48 470, 1969
12. Landis, E. M. & Pappenheimer J R. Exchange of substances through the capillary walls. Handbook of Physiology Sect. 2, vol. II, p. 961 Amer Physiological Society Washington, 1963
13 Lassen, N A. Muscle blood flow in normal man and in patients with intermittent claudication evaluated by simultaneous Xe^{133} and Na^{24} clearances. J Clin Invest 43 1805 1964.
14 Lassen, N A. On the theory of the local clearance method for measurement of blood flow including a discussion of its application to various tissues. Acta Med Scand Suppl. 47 136, 1967
15 Lees, M. H., Hill, J D Ochsner III A. J Thomas, C. L. & Novy M. J Maternal placental and myometrial blood flow of the rhesus monkey during uterine contractions. Amer J Obstet Gynec 110 68 1971
16. Lewis, B. V Uterine blood flow Obstet Gynec Survey 4 1211 1969
17 van Liew H. D. Semilogarithmic plots of data which reflect a continuum of exponential processes. Science 138 682, 1962.
18 Lundgren, O Blood flow distribution and counter current exchange in the small intestine Acta Physiol Scand, Suppl. 303 1967
19 Makowski, E. L., Meschia, G Droegemueller W & Battaglia, F C. Distribution of uterine blood flow in the pregnant sheep. Amer J Obstet Gynec 101 409 1968
20. Munck O Lysgaard, H., Pontonnier G., Lefèvre H. & Lassen, N A. Measurement of blood-flow through uterine muscle by local injection of ^{133}Xenon. Lancet I 14 1 1964
21 Nyström, C Forsman, L. & Roos, B. Myometrial blood flow studies n carcinoma of the corpus teri. Act Radiol (ther) 8 193, 1969
22. Prill, H J & Götz, F Blood flow in the myometrium and endometrium of the uterus. Amer J Obstet Gynec 82 10., 1961
23. Reynolds, S. R M M ternal blood flow in the uterus and placenta. Handbook of Physiology sect. -, vol. II, p. 1585 Amer Physiological Society Washington, 1963
24 Sejrsen, P & Tønnesen, K H Inert gas diffusion method for measurement f blood flow using saturation techniques. Circ Res 679 1968

Submitted for publication Nov 1 197

Lars Forsman
Department of Obstetrics and Gynecology
Östra sjukhuset
416 85 Göteborg
Sweden

Acta Obstet Gynec Scand 52: 335–343 1973

STIMULATION OF OVULATION WITH F-6066 CYCLOFENIL. BIS-(p-ACETOXYPHENYL)-CYCLOHEXYLIDENEMETHANE

Shimpei Tojo, Kaname Hayashi and Etsuji Osawa

From the Department of Obstetrics and Gynecology (Head: Prof. S. Tojo), Kobe University School of Medicine, Kobe, Japan

Abstract. Using animal experiments, the authors studied the mode of action of F 6066 (Cyclofenil) which is one of the chemical inducers of ovulation, and reached the following conclusions. F 6066 has dose-dependent effect, enhancing the actions of gonadotropin in the ovary. The activity of steroidogenic enzymes, such as Δ 3β-ol dehydrogenase, NAD- NADP-diaphorase and glucose-6-phosphate dehydrogenase, was activated directly by the administration of F 6066 in hypophysectomized experimental animals. Therefore F 6066 not only showed biological action on the hypothalamo-pituitary system promoting gonadotropin release, but also direct action on the ovary enhancing the response of the gonad to gonadotropins.

F 6066 (Bis-(p-acetoxyphenyl)-cyclohexylidene methane, Cyclofenil) a nonsteroidal estrogen preparation, has been clinically confirmed to be a potent stimulator of ovulation in anovulatory women. It is still obscure however where and in what manner the drug acts on the hypothalamo-pituitary-ovarian axis in order to induce ovulation.

This study was designed to demonstrate the mechanism of induction of ovulation by the drug and especially to investigate its direct action on the ovary. In some experiments clomiphene citrate (1 P-(β-diethylaminoethoxy)-phenyl-1, 2-diphenyl-2-chlor-ethylene), another estrogen preparation of the same category and possessing analogous properties, was also used to make a comparison of the biological action.

I. EFFECT OF F 6066 AND CLOMIPHENE ON THE OVARIAN RESPONSE TO EXOGENOUS GONADOTROPIN

A) *Material and methods*

Immature female rats of Sprague-Dawley strain provided by Japan Clea, Inc. (weighing 40–50 g) were hypophysectomized on the 22nd postnatal day. In order to study the effect on the ovarian response to exogenous follicle stimulating hormone (FSH), these animals were treated subcutaneously with 0.5 ml of solution in saline of 200 μg of NIH-FSH-P (its activity is equal to NIH-FSH-S$_1$) and 20 I.U. of human chorionic gonadotropin (HCG) as in the Steelman and Pohley ovarian augmentation assay in the morning and evening for 3 days starting from the 6th postoperative day. The treatment is shown in Fig. 1. At the same time, F 6066 or clomiphene was given to the same animals by the same route in different doses for 3 days. On the day after treatment all animals were sacrificed in order to weigh the ovary using a torsion balance. In order to estimate the ovarian response to exogenous luteinizing hormone (LH), other animals were treated with gonadotropin as in the Parlow ovarian ascorbic acid depletion assay before they underwent unilateral oophorectomy at 1 p.m. on the 5th day after the HCG injection. Then they received slow injection in a caudal vein of solution of 20 μg of NIH-LH-B$_1$ (its activity is equal to NIH-LH-S$_1$) in 0.5 ml of saline, and 4 hours later they were subjected to removal of the remaining ovary to study ovarian ascorbic acid depletion.

F 6066 or clomiphene was injected simultaneously with pregnant mare serum (PMS) in the doses for 3 consecutive days, as shown in Fig. 2.

In control experiment, three groups were divided, group untreated, group treated with 0. mg/kg/day estradiol and group treated with 1 mg/kg/day progesterone.

B) *Results*

A study of the change in ovarian response to FSH and HCG showed that there was marked increase in the weight of the ovary in the groups received 1 mg/kg/day or 10 mg/kg/day of F 6066 together with 200 μg of FSH, but there was no marked difference from the group treated with clomiphene. Nor was there any difference from the control groups receiving estradiol or progesterone.

In studying the ovarian response to LH, it was found that administration of 20 μg LH gave

Fig 1 Effect of F 6066 or clomiphene on ovarian augmentation after the administration of FSH (hypophysectomized rats in Sprague-Dawley strain).

rise to increased ovarian ascorbic acid depletion in a group treated with 10 mg/kg/day of F 6066 but no such effect with the other doses employed and no significant change was produced in groups given estradiol or progesterone in the above doses (Table I). Ovarian ascorbic acid depletion was significantly increased in a group treated with 0.1 mg/kg/day of clomiphene but rather inhibited in two groups treated with 0 05 mg/kg/day and 0.5 mg/kg/day respectively

II EFFECT OF F 6066 AND CLOMIPHENE ON OVULATION

A) *Materials and methods*

Immat re female Sprague Dawley rats (weighing 40-50 g) from J pan Clel Inc. were hypophysectomized intraauricularly on the 22nd postnatal day and 6 days later they received subcutaneous injection of 30 I.U of PMS and then at an interval of 56 hours 15 I.U f HCG, both dissolved in 0.5 ml saline. F 6066 or clomiphene was injected in the doses shown in Table for 5 days starting on the day before PMS i jection. 30 hours after

F g Effect f F 6066 or clomiphene on ovarian ascorbic acid depletion after the administration of LH (hypophysectomized rats in Sprague-Dawley strain).

Table I. *Effect of F 6066 or clomiphene on ovarian responsibility to the exogeneous gonadotrophes*

Hypophysectomized rats in Sprague-Dawley strain

A. Effect of F 6066 or clomiphene on ovarian augmentation with FSH

Treatment (mg/kg/day)		Total dose (mg)	No. of rats	NIH-FSH-P,200r + HCG Ovarian weight mg ± S.E.
Control		0	6	43.8 ± 5.7
Estradiol	0.2	0.6	5	37.6 ± 6.1
Progesterone	1.0	3.0	5	33.2 ± 4.1
F 6066	1.0	3.0	5	61.0 ± 9.5
	2.0	6.0	5	54.4 ± 1.3
	10.0	30.0	5	65.0 ± 5.1
CLOMID	0.05	0.15	5	46.4 ± 7.1
	0.1	0.3	5	38.0 ± 6.5
	0.5	1.5	4	40.0 ± 9.8

B. Effect of F 6066 or clomiphene on ovarian ascorbic acid depletion with LH

Treatment (mg/kg/day)		Total dose (mg)	No. of rats	NIH-LH-B,20r OAAD % ± S.E.
PMS HCG Control		0	5	28.4 ± 6.4
PMS HCG Estradiol	0.2	1.6	5	34.1 ± 3.2
PMS HCG Progesterone	1.0	8.0	5	32.5 ± 2.9
PMS HCG F 6066	1.0	8.0	5	37.7 ± 5.4
PMS HCG F 6066	2.0	16.0	5	39.0 ± 4.2
PMS HCG F 6066	10.0	80.0	5	41.6 ± 3.2
PMS HCG CLOMID	0.05	0.4	5	27.2 ± 4.2
PMS HCG CLOMID	0.1	0.8	5	44.8 ± 0.2
PMS HCG CLOMID	0.5	4.0	5	11.3 ± 3.0

the HCG injection all the animals were sacrificed to count the number of ova discharged in the fallopian tube and the uterus in order to study the effect of these compounds on ovulation. Groups receiving PMS and HCG alone served as controls. Ova were recovered by Miyake modification (8) of the method of Hopkins & Pincus (9) using an inverted microscope for the counting.

B) *Results*

A significant increase in the weight of the ovary was noted in the three groups receiving different doses of F 6066, 1 mg/kg/day 2 mg/kg/day and 10 mg/kg/day respectively and also in all groups treated with clomiphene. Increase in the weight of the uterus showed positive relationship with increase in dose in the groups receiving F 6066, but the increment of uterine weight was rather smaller with increasing doses of clomiphene (Table II).

Ovulation was induced in all cases receiving 30 I.U. PMS or 15 I.U. HCG. There was a marked increase in the number of ova discharged in

22 732464

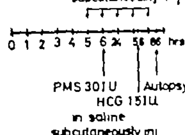
Table II *Effect of F 6066 or clomiphene on the induction of ovulation with PMS–HCG*

Hypophysectomized rats in Sprague–Dawley strain

Treatment	Total dose (mg)	Ovarian response	%	Average no. of ova (range)	Ovarian weight mg ± S.E.	Uterine weight mg ± S.E.
Control	—	7/7	100	7.9 (4–10)	44 1±4.3	61.0±5 1
F 6066						
0.2 mg/kg/D	1.0	6/6	100	4.3 (1 9)	37 7±5 1	87 7±4 6
1 0 mg/kg/D	5.0	8/8	100	11.5 (5–20)	65.6±3.7	98.8±7.2
2.0 mg/kg/D	10.0	10/10	100	10.5 (2 17)	64.9±4..	103 4±11 1
10.0 mg/kg/D	50 0	6/6	100	6.3 (4–8)	58 7±4 1	107.0±10.5
20 0 mg/kg/D	100.0	6/6	100	6.5 (6–7)	41 0±1 0	128.5±11 7
Estradiol benz.						
0.02 mg/kg/D	0.1	7/7	100	6.0 (6)	41.5±2.3	155.0±13.2
Clomid						
0.05 mg/kg/D	0.25	5/5	100	7 3 (5–11)	67.2±2.4	115.2±1 0
0.1 mg/kg/D	0.5	4/4	100	13.5 (9–16)	67 7±3.3	98.6±7 6
0.5 mg/kg/D	2.5	6/6	100	6.9 (4–10)	65.6±1 0	94.7±6.7

Treatment regimen.

Immature 22 day old female Sprague Dawley rat



groups given 1 mg/kg/day 2 mg/kg/day of F 6066 or 0 1 mg/kg/day of clomiphene as compared with the mean control count of 7 9

Incidentally the dose of clomiphene of 0 1 mg/kg/day is that which increased the LH-susceptibility of the ovary discussed earlier

III EFFECT OF F 6066 ON THE ACTIVITY OF THE OVARIAN STEROIDOGENIC KEY ENZYMES IN HYPO-PHYSECTOMIZED RAT

A) *Materials and methods*

Immature female Sprague-Dawley rats from Japan Clair Inc. with as uniform weights as possible were hypophysectomized intra-auricularly on the 22nd postnatal day and injected subcutaneously with 0.2 mg/kg (10 µg/rat), 1 mg/kg/(50 µg/rat), 2 mg/kg (100 µg/rat) or 20 mg/kg (1 000 µg/rat) of F 6066 dissolved in propylene glycol for 5 days starting from the 5th postoperative day

On the day after completion of the treatment the animals were sacrificed and immediately subjected to laparotomy to resect the ovaries, which were then weighed and refrigerated with dry ice before study

The ovaries thus obtained were stained histochemically with the following:

i) 3β-hydroxy steroid dehydrogenase (3β-HSD) (14)
ii) Glucose-6-Phosphate dehydrogenase (G-6-PDH) (9)
iii) NAD- and NADP-diaphorase (15)
NAD Nicotinamide-adenine dinucleotide
NADP Nicotinamide-adenine dinucleotide phosphate.

B) *Results*

1 *3β-HSD activity* On the 12th day after hypophysectomy the ovary was characterized by follicles having ceased to grow and mostly undergoing atresia with their granulosa cells showing a disorderly arrangement and with nuclear pyknosis and epithelial exfoliation in the follicles. The theca interna was thinned surrounded with atrophied interstitial gland cells At that time there was slightly positive 3β-HSD activity in the interstitial glands and the theca interna layer but the cells were apparently less active than in control animals of the same age

In groups receiving F 6066 the interstitial glands were found to be not so markedly atrophied and 3β-HSD activity in the theca interna and the gland was more than in the control group. The response tended to be intensified with the increase in dose of F 6066 as shown in Table III being especially marked at dosage levels of 100 µg/rat (2 mg/kg/day) and 1 000 µg/rat (20 mg/kg/day)

2 *G-6-PDH activity* In the hypophysectomized

control group, G-6-PDH activity was present mainly in the thinned theca interna and interstitial gland cells. As with 3β-HSD activity the G-6-PDH activity in the theca interna and especially in the interstitial glands was higher in animals received F 6066 than in the controls. Here again, the activity tended to be stronger at both sites with an increase in dose.

3. *NAD-diaphorase and NADP-diaphorase activities* In hypophysectomized control animals, the activity in the theca interna was significantly lowered compared with that in the interstitial glands. In groups receiving F 6066, by contrast, the activity in the theca interna and the interstitial glands was higher (like 3β-HSD and G-6-PDH activities) than in the control group at dosage levels of 100 μg and 1 000 μg (Table III).

IV. EFFECT OF F 6066 AND CLOMIPHENE ON ENDOGENOUS GONADOTROPIN SECRETION

A) *Material and methods*

In order to investigate the effect of F 6066 and clomiphene on the dynamics of pituitary gonadotropin secretion, immature female Sprague-Dawley rats were injected once subcutaneously with 30 I.U. PMS on the 21st postnatal day to induce ovulation. These animals received F 6066 or clomiphene by the same route for 4 consecutive days starting 2 days before PMS injection. F 6066 was administered in doses of 1 mg/kg/day, 2 mg/kg/day or 10 mg/kg/day and clomiphene 0.05 mg/kg/day, 0.1 mg/kg/day or 0.5 mg/kg/day. The effect of these compounds on the dynamics of gonadotropin secretion was investigated 48, 54 and 60 hours after PMS injection using the change in concentrations of hypophyseal and blood FSH and LH as indicators.

Groups of 10 rats were used in this study from which heparinized blood was collected from the jugular vein under ether anesthesia. The blood thus obtained was promptly centrifuged at 3 000 r.p.m. for 10 minutes to obtain the supernatant serum as test samples.

Immediately after decapitation, only the anterior lobe of the pituitary was isolated, refrigerated with dry ice, homogenized and then centrifuged (3 000 r.p.m., 10 min) in saline to obtain the supernatant fluid.

1.5 ml of the serum or the tissue corresponded to two and a half pituitaries was used as a sample for bioassay. LH activity was determined by Parlow ovarian ascorbic acid depletion method (10) and FSH activity by the ovarian augmentation test of Steelman–Pohley (17).

Table III. *Effect of F 6066 on the activity of enzymes in the ovary*

3-HSD DHA: Δ⁵-3β-ol dehydrogenase, DHA as a substrate. NAD diaph.: NAD-diaphorase. NADP diaph.: NADP-diaphorase. G-6-PD: glucose-6-phosphate dehydrogenase. Hypophysectomized rats in Sprague-Dawley Strain

	Group	3 HSD DHA	NAD diaph.	NADP diaph.	G-6-PD
Theca interna	Control				
	F 6066				
	10γ				
	50γ				
	100γ				
	1 000γ				
	Estradiol benzoate				
Interstitial tissue	Control	–			
	F 6066				
	10γ				
	50γ				
	100γ				
	1 000γ				
	Estradiol benzoate				

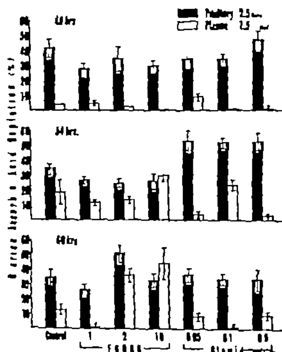


Fig. 3. Effect of F 6066 or clomiphene on the concentration of pituitary and serum LH after the subcutaneous injection of PMS (30 I.U.) (immature female rats in Sprague-Dawley strain). OAAD-method

B) *Results*

As shown in Fig. 3, there was a tendency for pituitary LH activity to decrease, with a marked elevation of blood LH activity 54 hours after administration of PMS alone. At this time pituitary FSH activity tended to rise while no substantial variation was caused in blood FSH activity.

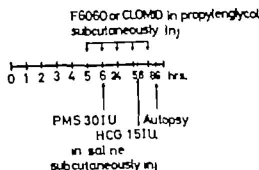
Table II *Effect of F 6066 or clomiphene on the induction of ovulation with PMS–HCG*

Hypophysectomized rats in Sprague–Dawley strain

Treatment	Total dose (mg)	Ovarian response	%	Average no. of ova (range)	Ovarian weight mg ± S E.	Uterine weight mg ± S.E.
Control	—	7/7	100	7.9 (4–10)	44.1±4.3	61.0±5.1
F 6066						
0.2 mg/kg/D	1.0	6/6	100	4.3 (1–9)	37 7±5 1	87 7±4 6
1 0 mg/kg/D	5.0	8/8	100	11.5 (5–20)	65 6±3 7	98.8±7.-
2.0 mg/kg/D	10.0	10/10	100	10.5 (2–12)	64 9±4.2	103 4±11 1
10.0 mg/kg/D	50 0	6/6	100	6.3 (4–8)	58 7±4 1	107.0±10.5
20.0 mg/kg/D	100.0	6/6	100	6.5 (6–7)	41.0±1 0	178.5±11 7
Estradiol benz.						
0 02 mg/kg/D	0.1	7/7	100	6.0 (6)	41.5±2.3	155.0±13.2
Clomid						
0.05 mg/kg/D	0.25	5/5	100	7.3 (5–11)	67.2±2.4	115.2±12.0
0.1 mg/kg/D	0.5	4/4	100	13.5 (9–16)	67 7±3.3	98.6±7.6
0.5 mg/kg/D	2.5	6/6	100	6.9 (4–10)	65 6±1.0	94 7±6.7

Treatment regimen.

Immature 22 day old female Sprague Dawley rat



groups given 1 mg/kg/day 2 mg/kg/day of F 6066 or 0.1 mg/kg/day of clomiphene as compared with the mean control count of 7 9

Incidentally the dose of clomiphene of 0 1 mg/kg/day is that which increased the LH-susceptibility of the ovary discussed earlier

III EFFECT OF F 6066 ON THE ACTIVITY OF THE OVARIAN STEROIDOGENIC KEY ENZYMES IN HYPOPHYSECTOMIZED RAT

A) *Materials and methods*

Immature female Sprague-Dawley rats from Japan Clair Inc. with as uniform weights as possible were hypophysectomized intra-auricularly on the 22nd postnatal day and injected subcutaneously with 0-- mg/kg (10 µg/rat), 1 mg/kg/(50 µg/rat) 2 mg/kg (100 µg/rat) or 20 mg kg (1 000 µg/rat) of F 6066 dissolved in propylene glycol for 5 days starting from the 5th postoperative day

On the day after completion of the treatment the animals were sacrificed and immediately subjected to laparotomy to resect the ovaries, which were then weighed and refrigerated with dry ice before study

The ovaries thus obtained were stained histochemically with the following:

i) 3β-hydroxy steroid dehydrogenase (3β-HSD) (14)
ii) Glucose-6-Phosphate dehydrogenase (G-6-PDH) (9)
iii) NAD- and NADP-diaphorase (15)
NAD Nicotinamide-adenine dinucleotide
NADP- Nicotinamide-adenine dinucleotide phosphate

B) *Results*

1 *3β-HSD activity* On the 1?th day after hypophysectomy the ovary was characterized by follicles having ceased to grow and mostly undergoing atresia with their granulosa cells showing a disorderly arrangement and with nuclear pyknosis and epithelial exfoliation in the follicles. The theca interna was thinned surrounded with atrophied interstitial gland cells. At that time there was slightly positive 3β-HSD activity in the interstitial glands and the theca interna layer but the cells were apparently less active than in control animals of the same age

In groups receiving F 6066 the interstitial glands were found to be not so markedly atrophied and 3β-HSD activity in the theca interna and the gland was more than in the control group. The response tended to be intensified with the increase in dose of F 6066 as shown in Table III being especially marked at dosage levels of 100 µg/rat (mg kg/day) and 1 000 µg/rat (?0 mg/kg/day)

- *G-6-PDH activity* In the hypophysectomized

marginal region of the anterior pituitary in lutein cells, interstitial cells and follicular cells of the ovary and in surface epithelia and stroma cells of the endometrium

DISCUSSION

J P Mayfield (7) studied the effect of clomiphene on the ascorbic acid content of the ovary on rats in state of pseudopregnancy and having found that ovarian ascorbic acid is decreased following administration of LH in low dose (0.02–0 6 mg/kg/day) but not in high doses (6 mg/kg/day 20 mg/kg/day) he concluded that clomiphene can enhance the sensitivity of the ovarian OAAD response to LH. Our results show that ascorbic acid depletion was increased by 0.1 mg/kg/day of clomiphene but inhibited at dosage level of 0 05 mg/kg/day or 0.5 mg/kg/day

I groups receiving F 6066, there was an increasing tendency to ovarian ascorbic acid depletion seen at a relatively high dosage level of 10 mg/kg/day a difference is apparently present between the two drugs with respect to effective dose.

It appears therefore that the optimum dose for sopentation of a gonadotropin effect in the ovary may vary for both drugs with the nature of the gonadotropin

The dose of estradiol of 0 1 mg/kg/day which served as control, was calculated of the basis of the estrogenicity of F 6066 on the uterus, and it as found that 1 mg of F 6066 is approximately equivalent to 1 µg of estradiol The fact that the increase in the eight of the uterus of F 6066-treated animals was comparable to that in the estradiol group suggests that the direct effect of F 6066 on the ovary may be specific enough biologically to depend wholly upon its estrogenicity

Early laboratory studies showed, on the one hand, that F 6066 has an inhibitory effect on the cornification of vaginal epithelium caused by estradiol benzoate According to Hohlkamp et al (4) on the other hand, clomiphene possesses eak estrogen effect together with potent antiestrogen activity

The result of the present study indicate that an increase in the weight of the ovary was brought about by F 6066 given in doses of 1 mg (0 mg/kg day and also 0 05 mg 0 5 mg/kg day of clomiphene and that 0.1 mg/kg/day of clomiphene could increase ovulation by intensifying the effect of gonadotropin in the ovary

F 6066 also increased ovulation at two different dosage levels 1 mg/kg/day and 2 mg/kg/day

In an in vitro study of the effect of F 6066 on the conversion of pregnenolone to progesterone or other Δ^4-3-ketosteroids, Larsson et al. (6) found the drug to be able to inhibit the conversion approximately 50% and concluded that a similar change may occur under in vivo conditions. This finding, however is not conclusive enough to determine whether F 6066 exerted a direct effect on 3β-HSD activity

This induced us to make the histochemical study discussed above, and it was found that the F 6066-treated group exhibited a higher activity than hypophysectomized controls, of 3β-HSD G-6-PDH and co-enzyme (i.e. NAD- and NADP diaphorase) in interstitial gland cells or theca interna cells of the ovary This activity was also found to rise in parallel with the increase in dose within the range referred to above. It was thus shown that the drug exerts an effect on steroidogenesis, acting directly on steroid-producing cells in the ovary

In immature male rats injected with 0 1 mg/kg/day 0 25 mg/kg/day or 0 5 mg/kg/day of clomiphene for 34 days, Roy et al. (11 12, 13) observed an increase in the weight of the prostate and seminal gland of these animals, which led them to assume an increased secretion of pituitary LH. As to the mechanism involved, they further presumed, in a study on immature female rats given radioactive estradiol, that clomiphene may prevent endogenous estrogen from binding, by competing with the latter at the receptors in the pituitary and hypothalamus

In our previous clinical trial of clomiphene and F 6066 we confirmed marked increase in urinary total gonadotropin or LH during, or immediately after the treatment Our present study shows that blood LH activity was significantly higher 60 hours after administration of 2 mg/kg/day or 10 mg/kg day of F 6066 than in controls, whereas FSH activity was increased in the pituitary but varied in the blood In groups receiving clomiphene, blood LH activity was increased earlier than in those treated with F 6066 54 hours after administration (0 1 mg/kg/day), and pituitary LH activity was also increasing. Thus, both

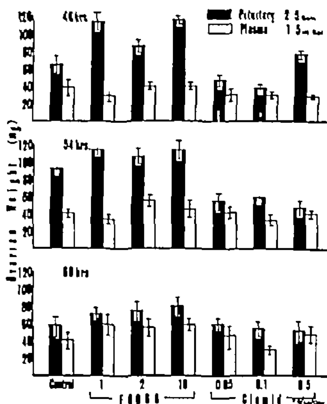


Fig 4 Effect of F 6066 or clomiphene on the concentration of pituitary and serum FSH after the subcutaneous injection of PMS (30 I U) (immature female rats in Sprague Dawley strain). Steelman-Pohley method.

Blood LH activity was markedly elevated 60 hours after treatment in the two groups receiving PMS along with 2 mg/kg/day and 10 mg/kg/day respectively of F 6066 as compared with a group treated with PMS alone. FSH activity tended to increase in the pituitary at 48 hours and between 54–60 hours in the groups receiving 1 mg/kg/day 2 mg/kg/day and 10 mg/kg/day of F 6066 respectively whereas it did not vary much in the blood. Blood LH activity was increased at 54 hours in a group given 0 1 mg/kg/day of clomiphene where LH activity in the pituitary also showed a tendency to increase No definite tendency was noted in FSH activity either in the pituitary or blood.

The drug seemed to produce lower FSH activity in the pituitary than F 6066 (Fig. 4).

V DISTRIBUTION OF ^{14}C F 6066 IN VARIOUS ORGANS

A) *Materials and methods*

^{14}C-labelled F 6066 was used as a tracer to study the distribution of the compound in various organs by the fluid scintillation technique. Tissues obtained by an identical procedure were examined by autoradiograph and at the same time studied histologically

The procedure consists of infusing as slowly as possible in a caudal vein of a pregnant mouse weighing 30 g 10.34 μc of ^{14}C F 6066 (specific activity 38.3 μg/mg) dissolved in 0.1 ml of a mixture of ethyl alcohol and 5% Tween 80. One hour later the animal was sacrificed to isolate organs immediately which were homogenized in 1 ml saline and then submitted to counting with hyamine-dioxane system in scintillator

For autoradiography isolated organs were immediately fixed in absolute alcohol to prepare paraffin sections (3 μ), which were dipped in Sakura autoradiographic emulsion (Emulsion type NR M No. 18 Konishiroku Photo Ind. Co, Ltd.). After 6 weeks exposure at 4°C in a dark case, the film was developed, fixed, washed in water and then stained with hematoxylin-eosin for microscopic observation.

B) *Results*

As shown in Fig. 5 a study of distribution of the radioactivity in various organs indicated that the radioactive intake per mg of tissue was largest for the pituitary which was followed by the liver ovary uterus and kidney in decreasing order It was shown that there was a considerable radioactive intake not only in central but peripheral target organs such as the ovary or uterus. Histological findings of autoradiograms demonstrated that radioactivity was present in β-cells in the

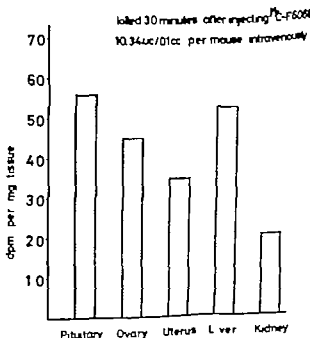


Fig 5 Distribution of ^{14}C F 6066 in various organs in pregnant mice

marginal region of the anterior pituitary in lutein cells, interstitial cells and follicular cells of the ovary and in surface epithelia and stroma cells of the endometrium.

DISCUSSION

J P Mayfield (7) studied the effect of clomiphene on the ascorbic acid content of the ovary on rats in a state of pseudopregnancy and having found that ovarian ascorbic acid is decreased following administration of LH in low dose (0.02–0.6 mg/kg/day) but not in high doses (6 mg/kg/day 20 mg/kg/day), he concluded that clomiphene can enhance the sensitivity of the ovarian OAAD response to LH Our results show that ascorbic acid depletion was increased by 0 1 mg/kg/day of clomiphene but inhibited at dosage level of 0 05 mg/kg/day or 0 5 mg/kg/day

In groups receiving F 6066, there was an increasing tendency to ovarian ascorbic acid depletion seen at a relatively high dosage level of 10 mg/kg/day a difference is apparently present between the two drugs with respect to effective dose

It appears therefore that the optimum dose for augmentation of a gonadotropin effect in the ovary may vary for both drugs with the nature of the gonadotropin.

The dose of estradiol of 0 1 mg/kg/day which served as control, was calculated of the basis of the estrogenicity of F 6066 on the uterus, and it was found that 1 mg of F 6066 is approximately equivalent to 1 µg of estradiol The fact that the increase in the weight of the uterus of F 6066-treated animals was comparable to that in the estradiol group suggests that the direct effect of F 6066 on the ovary may be specific enough biologically to depend wholly upon its estrogenicity

Early laboratory studies showed, on the one hand, that F 6066 has an inhibitory effect on the cornification of vaginal epithelium caused by estradiol benzoate According to Holtkamp et al (4) on the other hand, clomiphene possesses weak estrogen effect together with potent antiestrogen activity

The results of the present study indicate that an increase in the weight of the ovary was brought about by F 6066 given in doses of 1 mg – 10 mg/kg day and also 0 05 mg – 0 5 mg kg day of clomiphene and that 0.1 mg/kg/day of clomiphene could increase ovulation by intensifying the effect of gonadotropin in the ovary

F 6066 also increased ovulation at two different dosage levels: 1 mg/kg/day and 2 mg/kg/day

In an in vitro study of the effect of F 6066 on the conversion of pregenolone to progesterone or other Δ^4 3-ketosteroids, Larsson et al. (6) found the drug to be able to inhibit the conversion approximately 50% and concluded that a similar change may occur under in vivo conditions. This finding, however is not conclusive enough to determine whether F 6066 exerted a direct effect on 3β-HSD activity

This induced us to make the histochemical study discussed above and it was found that the F 6066-treated group exhibited a higher activity than hypophysectomized controls, of 3β-HSD G-6-PDH and co-enzyme (i e. NAD- and NADP diaphorase) in interstitial gland cells or theca interna cells of the ovary This activity was also found to rise in parallel with the increase in dose within the range referred to above. It was thus shown that the drug exerts an effect on steroidogenesis, acting directly on steroid-producing cells in the ovary

In immature male rats injected with 0 1 mg/kg/day 0 25 mg/kg/day or 0 5 mg/kg/day of clomiphene for 34 days, Roy et al (11 12 13) observed an increase in the weight of the prostate and seminal gland of these animals, which led them to assume an increased secretion of pituitary LH As to the mechanism involved they further presumed, in a study on immature female rats given radioactive estradiol, that clomiphene may prevent endogenous estrogen from binding, by competing with the latter at the receptors in the pituitary and hypothalamus

In our previous clinical trial of clomiphene and F 6066 we confirmed a marked increase in urinary total gonadotropin or LH during, or immediately after the treatment. Our present study shows that blood LH activity was significantly higher 60 hours after administration of 2 mg/kg/day or 10 mg/kg day of F 6066 than in controls, whereas FSH activity was increased in the pituitary but varied in the blood. In groups receiving clomiphene blood LH activity was increased earlier than in those treated with F 6066, 54 hours after administration (0 1 mg/kg/day), and pituitary LH activity was also increasing. Thus, both

F 6066 and clomiphene caused an increase in blood LH activity with an increase in pituitary LH activity although to a different extent both in time and in degree This finding provides evidence that these compounds have the property of LH releasers or promoters of LH release by acting on the hypothalamus.

Using oophorectomized mature Wistar rats, Taubert, Kessler et al. (18) also investigated the effect of F 6066 and clomiphene on LH RF at hypothalamic level, and found that LH RF was lowered at *dosage levels of 100–600 μg/rat of* F 6066 or 25 μg/rat of clomiphene and that the decrease was more marked with F 6066 just as was in our experience.

According to Hanngren Elner Jensen et al. (2) who carried out detailed laboratory studies by means of [14]C F 6066 for whole body autoradiography the lutein tissue of the ovary and yolk sac were the only organs, except the liver where the administration of the drug was rapidly followed by its selective accumulation in the organs, with its uptake still persisting 24 hours later It was also reported that the drug is excreted soon after injection mostly via the kidney and partly from the liver into the bile and then finally into the feces through the intestinal tract, where the partial reabsorption in the entero-hepatic circulation occurs.

This corresponds well with our finding that there was high radioactivity detectable in the liver and rather low in the kidney

Hanngren further stated that a considerable amount of radioactivity was also seen from 5 to 60 minutes after the injection in the brain particularly a fairly high concentration in the grey matter and in the pituitary for 4 hours after the injection.

Uptake of the drug was noted from 5 minutes after the injection in the mouse ovary particularly in the corpus luteum gravidarum reached a peak at 20 minutes and then rapidly decreased, although it was still detectable 24 hours later

In non-pregnant mice by contrast the radioactivity was very high in some corpora lutea and very low in others, while there was a greater uptake of the drug in interstitial cells than in mature follicles.

However the uptake was extremely slight in the endometrium. Our results both of fluid scintillation study and autoradiography indicate by contrast, a considerable uptake of the drug in the uterus as well as the anterior pituitary and ovary

CONCLUSION

The following conclusions may be drawn from the studies about the mode of action of F 6066 and clomiphene.

1 Both F 6066 and clomiphene have a dose dependent effect which enhances gonadotropin action in the ovary acting directly on the ovary It appears that the *optimum dose for such an effect* varies according to the nature of the exogenous gonadotropin.

2. The activity in the ovary of *3β-HSD G-6-PDH NAD- and NADP-diaphorase was higher* in hypophysectomized rats receiving F 6066 than that in the control.

3 In rats with ovulation induced by PMS, *F 6066 increased FSH in the pituitary and LH* release into the blood, while clomiphene also acted as an LH releaser although its effect was weaker and manifested earlier than that of F 6066.

4 The distribution pattern of [14]C F 6066 in various organs showed that the drug is taken up to a marked extent not only in central but also in peripheral organs, particularly in the ovary and the uterus. This supports the assumption that the drug may act directly on the ovary

ACKNOWLEDGEMENTS

The authors are grateful to D Leideman of Ferrosan Co. Ltd., Sweden and D K. Sato of Teikoku Hormone MFG Co. Ltd. for their supply of F 6066. The authors are grateful also to Dr R W Bates (NIAMD NIH, USA) for his kind supply of gonadotropin preparations.

REFERENCES

1 Background Information on Compound *F 6066* Medical Dept., AB Ferrosan, Malmö, Sweden, 1965
Hanngren, A., Einer Jensen, N & Ullberg, S Nature 205. 461–46_, 1965
3 — Acta Endoc 40 35–43, 1965
4. Holtkamp, D E., Greslin, J G Root, C. A. & Lerner L J Proc Soc Exper Biol Med *105* 197 1960.
5 Hopkins, T F & Pincus, G Endocrinology *73* 775 1963
6. Larsson, H & Stensson, M Acta Endocr 45 673–684 1963
7 Mayfield, J D & Ward, D N Act Endocr *51* 557 562, 1966
8. Miyake T Kobayashi, H. & Hara, K., Folia Endocr Japon 4 357 1966 (in Japanese).

9 Nachlas, M. M., Walker, D. G & Seligman, A. M. J Biophys Biochem Cytol 4 169 1958.
10. Parlow A. F Fed Proc 17 402, 1958.
11 Roy S., Mahesh, V B. & Greenblatt, R. B. Acta Endocr 47 645 1964.
12 — Acta Endocr 47 657 1964
13 — Acta Endocr 47 669 1964.
14 Rubin, B L., Leipsner G. & Deane, H. W Endocrinology 69 619 1961.
15. Scarpelli, D G Hess, R. & Pearse, A. G. E., J Biophys Biochem Cytol 4 747 1958.
16. Schomer, F A. & Nast H Nature 194 154, 1962.
17. Steelman, S. L. & Pohley F M Endocrinology 53 604, 1953
18. Timbert, H. D Kessler, R., Bach, G & Werner, H. J Experientia 26 97 1970
19 Wattenberg, L. W J Histochem Cytochem 6 225 1958

Submitted for publication Nov 27 1972

Shinpei Tojo
Department of Obstetrics and Gynecology
Kobe University School of Medicine
Ikutaku
Kobe 650
Japan

Acta Obstet Gynec Scand 52, 345–354 1973

FREE ZONE ELECTROPHORESIS OF AMNIOTIC FLUID IN NORMAL PREGNANCIES AND IN PREGNANCIES COMPLICATED BY HAEMOLYTIC DISEASE

Lars-Erik Jonasson and Stellan Hjertén

From the Department of Obstetrics and Gynaecology, University Hospital (Head, Professor Carl Gemzell) and the Institute of Biochemistry (Head, Professor Stellan Hjertén) University of Uppsala, Uppsala, Sweden

Abstract. Sixty-four samples of undialysed amniotic fluid were analysed in a free zone electrophoresis apparatus equipped with an ultra-violet (UV) scanning attachment for quantitative determinations of the protein concentrations. Total protein concentrations were measured by biuret method. The electrophoretic patterns of amniotic fluid were generally simpler than those from maternal or cord serum or plasma. The albumin fraction showed the highest correlation to total protein levels in amniotic fluid both in normal pregnancies and in pregnancies complicated by haemolytic disease. Samples from pregnancies complicated by moderate to serious haemolytic disease showed a tendency to a more rapid decrease of the main protein fractions during the later phase of pregnancy. No significant connections could be found between the main protein fractions and cord blood haemoglobin or bilirubin concentrations. A probably significant correlation existed between the values for the albumin fraction and the values for amniotic fluid bile pigments (ΔE₄₅₀) in normal pregnancies but not in pregnancies with haemolytic disease. A high nonprotein peak in the patterns from amniotic fluid was found to consist mainly of uric acid. The size of this peak was positively correlated with infant birth weight and negatively correlated with concentrations of albumin and total protein in normal pregnancies. Polyacrylamide gel gradient electrophoresis of some samples showed amniotic fluid to be rich in proteins with relatively low molecular weights.

Studies of the total protein concentrations in the amniotic fluid have shown differences in concentrations between some clinical groups (14). The present study is an attempt to evaluate further the variation of the main protein components against clinical data in a series of normal pregnancies and pregnancies complicated by Rh-immunization. Special interest was centered around the albumin content as this protein serves as the main carrier for several low-molecular components, for instance bilirubin (18, 25) and also possesses other important physiological properties.

In the present investigation free zone electrophoresis was employed, a technique which has not been used before in the study of amniotic fluid. Paper electrophoresis has been utilized by several authors (1, 8, 22), electrophoresis in strips of cellulose acetate by Wild (26) and in a polyacrylamide gel by Usategui-Gomez et al. (23), Fishbacher & Quinlivan (6). In general the series are rather small and most authors have failed to demonstrate any constant pattern typical for pregnancies complicated by haemolytic disease.

MATERIAL

Fifteen samples of amniotic fluid were taken by abdominal amniocentesis from 15 patients admitted for therapeutic abortion. The duration of these pregnancies was 12 to 21 weeks. According to ordinary clinical criteria these pregnancies were normal.

Twenty-four samples came from 22 pregnancies in the 33rd–43rd week. The majority of the patients were admitted because of suspected Rh-immunization but the actual pregnancy resulted in an Rh-negative and healthy child with a negative direct Coombs' test on cord blood. These samples were taken by abdominal amniocentesis. Some samples were also collected during elective caesarean section for contracted pelvis. All these patients were considered to represent normal pregnancies.

Twenty-five samples were taken from 22 Rh-immunized patients with affected fetuses. All these infants were Rh-positive and had a positive direct Coombs' test on cord blood. The samples were taken by abdominal amniocentesis in the 32nd to the 37th week. These patients were divided into two subgroups. The first contained cases with mild haemolytic disease and a cord blood haemoglobin value of 12.1 g/100 ml or higher. As pointed out previously this haemoglobin value may be considered

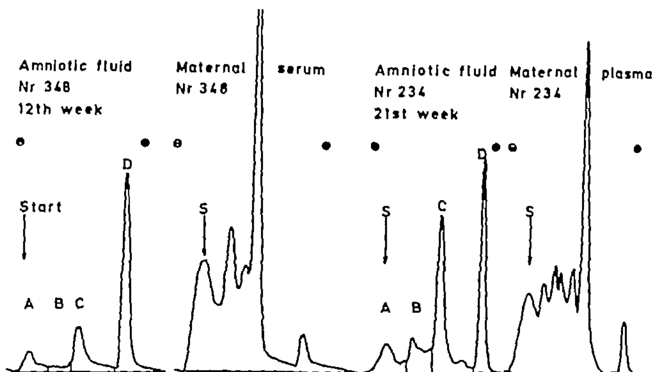


Fig. 1. Free zone electrophoretic patterns from two normal pregnancies in the first and second trimester. A, B, C and D are the main electrophoretic fractions.

to be the lower limit of normal in this series (15). The second subgroup involved cases with moderate to severe haemolytic disease. The cord blood haemoglobin values in this subgroup were in the range of 9.4–5.4 g/100 ml. One infant died postnatally of haemolytic disease but the series contained no case of hydrops fetalis. The first subgroup consisted of 15 samples from 14 patients, the second of 10 samples from 8 patients.

The technique for amniocentesis and the handling of the samples was described previously (13). Samples from patients with doubtful menstrual data and samples contaminated by blood or meconium were not included in this study. None of the patients were in labour. In most cases samples of maternal venous blood were taken the same day as amniocentesis. In some cases it was also possible to obtain samples of cord blood. The samples of amniotic fluid and plasma or serum were stored frozen at −18°C.

METHODS

Free zone electrophoresis was performed according to the method described by Hjertén (10, 11). As the name of the method implies no anticonvection agent is included in the electrophoresis tube. Stabilization against convection is achieved instead by a slow rotation of the tube around its long axis (40 rpm). For detection and quantitative measurements of proteins and other UV absorbing substances the electrophoresis tube is scanned during the run with the aid of a rotating filter transmitting light of the wavelengths 280 and 320 nm. The ratio between the transmissions of these two wavelengths is fed into a recorder which guarantees a smooth base line.

The buffer used was 0.1 M Tris–acetic acid, pH 8.0. The sample volume used was about 0.007 ml. The current was adjusted to 5 mA corresponding to a voltage of 700 volts. The running time in most experiments was 40 minutes. Calibration curves were constructed from readings of known serum dilutions to correct for lack of proportionality between the amount of protein in a zone in the electrophoresis tube and the area of the corresponding peak on the recorder chart.

Using the calibration curves the relative corrected area of each peak was calculated for each sample of amniotic fluid and the proportions thus determined applied to the total protein content of each sample. The total protein content was determined by a biuret method (14). The measurements of peak areas was made by planimetry. The coefficient of variation for the planimetry of the individual peaks at six repeated determinations of the same sample ranged ± 6–5.5%. The samples were not pretreated in any way and only the peaks corresponding to proteins were used in the calculations.

A few samples were studied with electrophoresis in a gradient of a polyacrylamide gel (12). Such a gradient separated proteins according to molecular size. The gel had the dimensions 0 7 9 cm. The gradient which was linear went from total concentration T of 30 down to 5%. The crosslinking concentration C was constant throughout the gradient and equal to 3. The buffer used was 0.1 M Tris–acetic acid, pH 8.5. The run was conducted for 17 hours at 150 volts. Nondialysed amniotic fluid was used in a volume of 0.2 ml and plasma or serum in a volume of 0.005 ml.

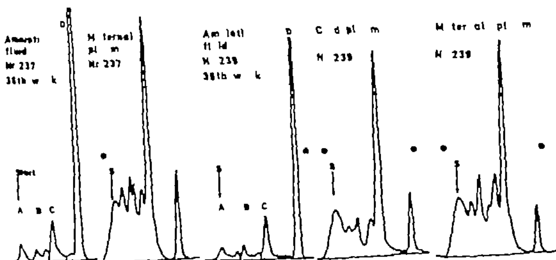


Fig. 2. Free zone electrophoretic patterns from normal pregnancy (No. 237) and pregnancy complicated by haemolytic disease and an infant's cord blood haemoglobin value of 5.4 g/100 ml (No. 239).

To isolate nonprotein components with high ultraviolet light absorption three samples of amniotic fluid were treated separately by preparative electrophoresis in an agarose suspension (9) and the fractions of interest were analysed for amino acids by the ninhydrin reaction and for uric acid and creatinine by the routine methods of the hospital laboratory (7).

Experiments with dilutions of uric acid in the apparatus for free electrophoresis showed UV light absorption to be proportional to uric acid concentrations under the conditions used in the present study.

Estimation of bile pigments (ΔE_{450}) in the amniotic fluid was made according to the method devised by Liley (17).

RESULTS

General appearance of the electrophoretic patterns

Amniotic fluid patterns showed only small differences between the clinical groups. The patterns of amniotic fluid were always simpler than corresponding patterns of maternal and fetal serum or plasma. Typical examples of patterns from early and late normal pregnancies are presented in Figs 1 and . A typical pattern from a pregnancy complicated by serious haemolytic disease is shown in Fig. . In most cases three main protein peaks (A, B and C) were clearly visible and in front of them a big nonprotein peak (D) which was less pronounced in samples of maternal and fetal serum or plasma. The material corresponding to this peak passed the pores of a dialysis bag. When separated by preparative electrophoresis in an agarose suspension this peak gave a negative ninhydrin reaction. Its spectral curve and electrophoretic mobility was similar to that of uric acid (Fig. 3). Chemical analysis (7) confirmed the presence of uric acid. In one of the three samples analysed the uric acid fraction contained traces of creatinine.

Relative distribution of the protein fractions

The mean values for the corrected areas of the protein fractions were calculated for two-week periods. The results were expressed as percentages of the total protein area.

The relative size of fraction A was small during periods with a high total protein content, around the 20th week in patients with normal pregnancies, and then obtained a higher and constant value during the later phase of pregnancy (Tables I and II).

Fraction B was relatively small during early pregnancy and then showed a somewhat higher and constant level during the later phase of normal pregnancies (Tables I and II).

Fraction C in normal patients showed an increase up to the 18th–21st week and decrease during the later phase of pregnancy (Tables I and II).

The two groups of isoimmunized patients with affected fetuses showed the same general pattern

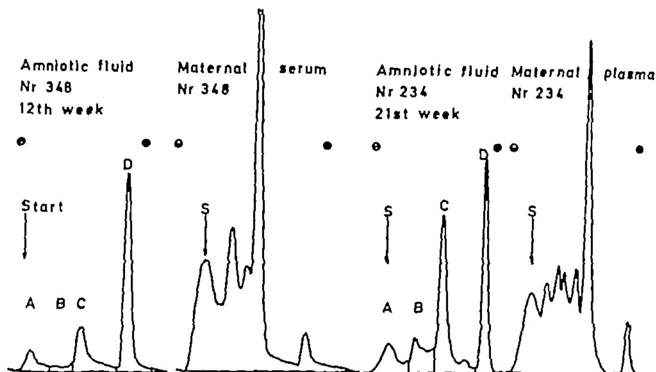


Fig 1 Free zone electrophoretic patterns from two normal pregnancies in the first and second trimester A, B, C and D are the main electrophoretic fractions.

to be the lower limit of normal in this series (15) The second subgroup involved cases with moderate to severe haemolytic disease. The cord blood haemoglobin values in this subgroup were in the range of 9 4–5 4 g/100 ml. One infant died postnatally of haemolytic disease, but the series contained no case of hydrops fetalis. The first subgroup consisted of 15 samples from 14 patients, the second of 10 samples from 8 patients.

The technique for amniocentesis and the handling of the samples was described previously (13). Samples from patients with doubtful menstrual data and samples contaminated by blood or meconium were not included in this study None of the patients were in labour In most cases samples of maternal venous blood were taken the same day as amniocentesis. In some cases it was also possible to obtain samples of cord blood. The samples of amniotic fluid and plasma or serum were stored frozen at −18°C.

METHODS

Free zone electrophoresis was performed according to the method described by Hjertén (10, 11) As the name of the method implies no anticonvection agent is included in the electrophoresis tube. Stabilization against convection is achieved instead by a slow rotation of the tube around its long axis (40 rpm). For detection and quantitative measurements of proteins and other UV absorbing substances the electrophoresis tube is scanned during the run with the aid of a rotating filter transmitting light of the wavelengths 280 and 320 nm. The ratio between the transmissions of these two wavelengths is fed into a recorder which guarantees a smooth base line.

The buffer used was 0.1 M Tris-acetic acid, pH 8 0. The sample volume used was about 0.007 ml. The current was adjusted to 5 mA corresponding to a voltage of 700 volts. The running time in most experiments was 40 minutes. Calibration curves were constructed from readings of known serum dilutions to correct for lack of proportionality between the amount of protein in a zone in the electrophoresis tube and the area of the corresponding "peak" on the recorder chart.

Using the calibration curves the relative corrected area of each peak was calculated for each sample of amniotic fluid and the proportions thus determined applied to the total protein content of each sample. The total protein content was determined by a biuret method (14). The measurements of peak areas was made by planimetry The coefficient of variation for the planimetry of the individual peaks at six repeated determinations of the same sample ranged ±2 6–5.5% The samples were not pretreated in any way and only the peaks corresponding to proteins were used in the calculations.

A few samples were studied with electrophoresis in a gradient of a polyacrylamide gel (12) Such a gradient separated proteins according to molecular size The gel had the dimensions 0 2 7 9 cm The gradient which was linear went from total concentration T of 30% down to 5% The crosslinking concentration C was constant throughout the gradient and equal to 3%. The buffer used was 0 1 M Tris-acetic acid, pH 8.25 The run was conducted for 17 hours at 150 volts. Nondialyzed amniotic fluid was used in volume of 0.. ml and plasma or serum in a volume of 0 005 ml.

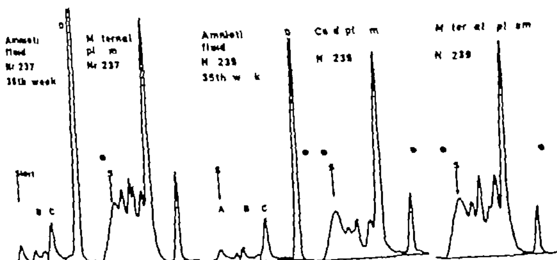


Fig. 2. Free zone electrophoretic patterns from normal pregnancy (No. 237) and pregnancy complicated by haemolytic disease and an ultimate cord blood haemoglobin value of 5.4 g/100 ml (No. 239).

To isolate nonprotein components with high ultraviolet light absorption three samples of amniotic fluid were treated separately by preparative electrophoresis in an agarose suspension (9) and the fractions of interest were analysed for amino acids by the ninhydrin reaction and for uric acid and creatinine by the routine methods of the hospital laboratory (7).

Experiments with dilutions of uric acid in the apparatus for free electrophoresis showed UV light absorption to be proportional to uric acid concentrations under the conditions used in the present study.

Estimation of bile pigments (ΔE_{450}) in the amniotic fluid was made according to the method devised by Liley (17).

RESULTS

General appearance of the electrophoretic patterns

Amniotic fluid patterns showed only small differences between the clinical groups. The patterns of amniotic fluid were always simpler than corresponding patterns of maternal and fetal serum or plasma. Typical examples of patterns from early and late normal pregnancies are presented in Figs. 1 and 2. A typical pattern from a pregnancy complicated by serious haemolytic disease is shown in Fig. 2. In most cases three main protein peaks (A, B and C) were clearly visible and in front of them a big nonprotein peak (D) which was less pronounced in samples of maternal and fetal serum or plasma. The material corresponding to this peak passed the pores of a dialysis bag. When separated by preparative electrophoresis in an agarose suspension this peak gave a negative ninhydrin reaction. Its spectral curve and electrophoretic mobility was similar to that of uric acid (Fig. 3). Chemical analysis (7) confirmed the presence of uric acid. In one of the three samples analysed the uric acid fraction contained traces of creatinine.

Relative distribution of the protein fractions

The mean values for the corrected areas of the protein fractions were calculated for two-week periods. The results were expressed as percentages of the total protein area.

The relative size of fraction A was small during periods with a high total protein content, around the 20th week in patients with normal pregnancies, and then obtained a higher and constant value during the later phase of pregnancy (Tables I and II).

Fraction B was relatively small during early pregnancy and then showed a somewhat higher and constant level during the later phase of normal pregnancies (Tables I and II).

Fraction C in normal patients showed an increase up to the 18th–21st week and a decrease during the later phase of pregnancy (Tables I and II).

The two groups of nonimmunized patients with affected fetuses showed the same general pattern

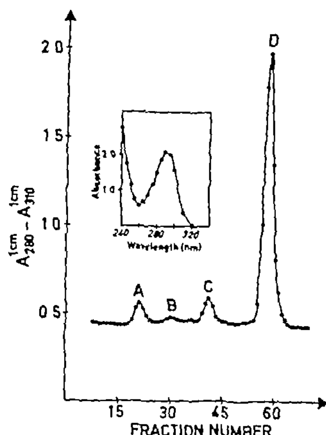


Fig. 3 Preparative agarose suspension electrophoresis of amniotic fluid. The experiment was performed in 0.1 M Tris-HCl, pH 8.0 at a current of 63 mA. The inner diameter of the electrophoresis tube: 2 cm. Duration of the run: 1.5 hours. Sample volume: 3 ml amniotic fluid. The agarose concentration: 0.17%. The absorption spectrum of the material corresponding to fraction D is inserted.

(Table II). The differences between the groups were not significant except for a probably significantly elevated value of fraction C in patients with *slight haemolytic disease during the 35th–36th week* compared with the normals ($0.05 > p > 0.01$).

Absolute concentrations of the protein fractions

The *main protein fractions* showed a rapid increase during the early phase of normal pregnancies (Table I). *During the later stage of normal* pregnancies the values for fractions A and B *were constant or slowly* decreasing while fraction C showed a rapid decrease (Table III and Fig. 4). The shifts in concentrations between the two-week periods during normal late pregnancies were only significant for fraction B between the 35th–36th and 41st–43rd week ($0.01 > p > 0.001$).

Comparing normal patients with the two groups of patients with haemolytic disease *a tendency* to elevated values for fraction C appeared in the group with slightly affected fetuses and a tendency to rapid decrease of the same fraction in the group with serious disease. Fraction B showed *a probably significantly lowered value for the* seriously affected group in the weeks 35–36 ($0.05 > p > 0.01$). Otherwise the differences between the clinical groups were not significant (Table III).

The individual concentrations for normal late pregnancies and for the two groups with haemolytic disease are given in Fig. 4. Regression lines calculated for the protein fractions showed no significant differences in slope between normal *patients and patients with slight* haemolytic disease. In isoimmunized patients with anaemic infants all three fractions showed a more pronounced downward trend. The slope for fraction A *was significantly greater in this group compared*

Table I *Relative and absolute values for the three main protein fractions A, B and C and the nonprotein fraction D during the early phase of normal pregnancies. Means ± S.E.M.*

Week of pregnancy	n	Total protein content (mg/100 ml)	Electrophoretic fraction A %	Electrophoretic fraction A mg/100 ml	Electrophoretic fraction B %	Electrophoretic fraction B mg/100 ml	Electrophoretic fraction C %	Electrophoretic fraction C mg/100 ml	Electrophoretic fraction D (area mm²)
12–13	1	138	29.0	40.0	9.2	12.7	61.8	85.3	151.6
14–15	3	250.0	28.1	62.6	8.8	25.0	63.1	16[illegible]	151.9
		±73.5	±5.5	±11.3	±2.7	±11.0	±4.6	±51.7	±45.2
16–17	3	325.0	18.4	59.6	19.2	64.1	62.4	203.3	123.3
		±34.0	±0.6	±5.1	±2.6	±9.3	±2.3	±4.7	±9.6
18–19	1	948	12.1	114.7	13.8	130.8	74.1	702.5	95.0
20–21	7	61[illegible].0	14.3	86.4	16.8	100.3	68.9	425.3	135.2
		±45.7	±1.2	±6.6	±2.3	±11.9	±2.4	±43.8	±4.8

Table II *a–c*. *Relative distribution of the main electrophoretic fractions A, B and C in percent of the total area*

Mean ± S.E.M. Normal pregnancies and pregnancies with haemolytic disease. The haemoglobin values refer to cord blood haemoglobin. Figures within brackets denote number of samples

Week of pregnancy	Normal pregnancies	Haemolytic disease (Hb > 12.1 g/100 ml)	Haemolytic disease (Hb < 10.0 g/100 ml)
(a) *Electrophoretic fraction A %*			
31–32	—	—	17.9 (1)
33–34	20.7 ± 2.4 (3)	15.2 ± 2.3 (4)	20.6 ± 2.3 (4)
35–36	22.3 ± 1.7 (4)	20.0 ± 0.6 (6)	24.2 ± 3.3 (4)
37–38	23.8 ± 2.4 (6)	24.1 ± 2.4 (5)	14.5 (1)
39–40	25.8 ± 3.9 (7)	—	—
41–43	26.3 ± 4.4 (4)	—	—
(b) *Electrophoretic fraction B %*			
31–32	—	—	15.9 (1)
33–34	18.5 ± 3.3 (3)	19.7 ± 1.8 (4)	19.0 ± 1.9 (4)
35–36	24.3 ± 1.7 (4)	20.1 ± 1.8 (6)	20.5 ± 1.4 (4)
37–38	20.5 ± 2.7 (6)	20.2 ± 0. (5)	18.3 (1)
39–40	20.2 ± 1.3 (7)	—	—
41–43	21.2 ± 0.3 (4)	—	—
(c) *Electrophoretic fraction C*			
31–32	—	—	66.3 (1)
33–34	40.8 ± 4.2 (3)	65.1 ± 3.6 (4)	60.3 ± 3.7 (4)
35–36	53.3 ± 1.9 (6)	59.9 ± 1.9 (6)	55.3 ± 4.5 (4)
37–38	53.7 ± 3.7 (6)	55.6 ± 6 (5)	67.2 (1)
39–40	54.0 ± 2.2 (7)	—	—
41–43	52.5 ± 4.1 (4)	—	—

both with the normal cases and with the isoimmunized cases without fetal anaemia.

Polyacrylamide gradient electrophoresis

This method (1) resulted in a very high resolution. A low content of proteins with large molecules was common to all amniotic fluid samples examined (Fig. 5). Proteins of intermediate size seemed to be represented in higher concentrations in samples from pregnancies complicated by haemolytic disease but these samples also had high total protein content. Amniotic fluid seemed to be rich in components with low molecular weight as in front of the albumin band 4–7 bands appeared compared with 2 or 3 in serum (Fig. 5).

Table III *a–c*. *Mean values in mg/100 ml ± S.E.M for the electrophoretic fractions A, B and C*

Normal pregnancies and pregnancies with haemolytic disease. The haemoglobin values refer to cord blood haemoglobin. Figures within brackets denote number of samples

Week of pregnancy	Normal pregnancies	Haemolytic disease Hb > 12.1 g/100 ml	Haemolytic disease Hb < 10.0 g/100 ml
(a) *Electrophoretic fraction A*			
31–32	—	—	85.9 (1)
33–34	55.0 ± 4.6 (3)	48.4 ± 13.1 (4)	59.7 ± 4.4 (4)
35–36	64.2 ± 8.2 (4)	65.0 ± 10.5 (6)	64.8 ± 6.4 (4)
37–38	59.9 ± 4.6 (6)	63.2 ± 5.8 (5)	25.2 (1)
39–40	56.9 ± 6.2 (7)	—	—
41–43	45.5 ± 4.8 (4)	—	—
(b) *Electrophoretic fraction B*			
31–32	—	—	76.3 (1)
33–34	48.5 ± 5.8 (3)	57.9 ± 13.0 (4)	55.3 ± 3.3 (4)
35–36	69.3 ± 5.8 (4)	65.7 ± 12.3 (6)	55.9 ± 2.6 (4)
37–38	50.0 ± 10.0 (6)	55.5 ± 5.2 (5)	31.8 (1)
39–40	48.1 ± 7.2 (7)	—	—
41–43	39.4 ± 6.5 (4)	—	—
(c) *Electrophoretic fraction C*			
31–32	—	—	318.2 (1)
33–34	168.5 ± 35.5 (3)	186.7 ± 27.4 (4)	185.1 ± 50.4 (4)
35–36	153.7 ± 16.2 (4)	194.2 ± 22.0 (6)	151.2 ± 20.8 (4)
37–38	130.5 ± 18.3 (6)	157.3 ± 26.4 (5)	116.9 (1)
39–40	125.1 ± 14.9 (7)	—	—
41–43	99.6 ± 22.7 (4)	—	—

The nonprotein fraction D

The area of fraction D showed a slow increase during the course of late normal pregnancy and

Table IV. *Mean values ± S.E.M. for total protein and for the nonprotein electrophoretic fraction D*

Normal pregnancies and pregnancies complicated by haemolytic disease. Figures within brackets indicate number of samples

Week of pregnancy	Total protein mg/100 ml			Electrophoretic fraction D area mm²		
	Normals	Cord haemoglobin (≥12.1 g/100 ml)	Cord haemoglobin (<10.0 g/100 ml)	Normals	Cord haemoglobin (≥12.1 g/100 ml)	Cord haemoglobin (<10.0 g/100 ml)
31–32	—	—	480	—	—	158.8
33–34	272.0±37.3 (3)	291.0±49.6 (4)	300.0±32.0 (4)	303.4±14.1 (3)	200.1±25.9 (4)	187.6±5.1 (4)
35–36	287.3±23.8 (4)	325.0±53.4 (6)	73.8±14.7 (4)	258.4±20.4 (4)	203.5±17.4 (6)	197.4±9.0 (4)
37–38	240.3±26.7 (6)	276.0±79.0 (5)	174 (1)	229.7±16.7 (6)	266.5±17.0 (5)	235.0 (1)
39–40	230.1±22.0 (7)	— —	— —	320.8±39.9 (7)	— —	— —
41–43	184.4±27.3 (4)	—	—	348.4±51.6 (4)	—	—

was considerably larger than during early pregnancy (Tables I and IV). The shifts in mean area during the course of normal late pregnancy were not significant. The difference in mean area between normal patients and patients with slight haemolytic disease was probably significant during the 33rd–34th week ($0.05 > p > 0.01$) and highly significant ($0.001 > p$) between normal patients and patients with serious haemolytic disease for the same period. In the 35th–36th week a probably significant difference existed between normal patients and patients with serious disease ($0.05 > p > 0.01$).

During early pregnancy fraction D showed no

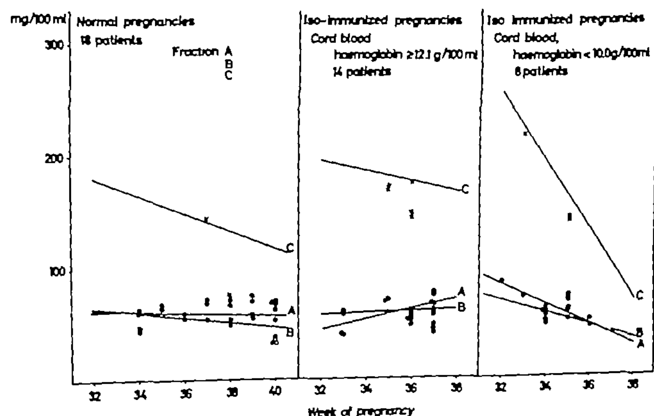


Fig. 4. Individual values and regression lines for the main protein fractions. Designations same as in Figs. 1 and 2. Normal pregnancies and pregnancies with slight and serious haemolytic disease.

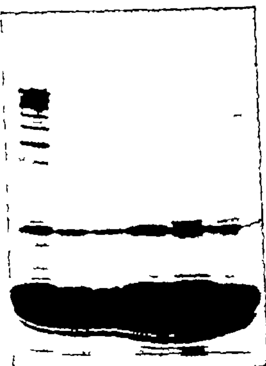


Fig 5 Polyacrylamide gradient electrophoresis. From the left sample of normal adult serum, two normal amniotic fluids (40th week), amniotic fluid from case with serious haemolytic disease (33rd week), amniotic fluids from two cases with slight haemolytic disease (34th and 37th week)

correlation to total protein content of amniotic fluid or to any of the protein fractions. In the later phase of normal pregnancies (33 d–43rd week n 24) fraction D showed probably significant negative correlations both with the total protein content and with the values for fractions B and C ($r = -0.47$ -0.45 and 0.44 0.05 p 0.01)

Isoimmunized patients with slight haemolytic disease also showed probably significant negative correlation between the values for fraction D and the concentrations of total protein, fraction B and fraction C ($n = 15$ $= -0.57$ -0.58 and 0.57 0.05 p 0.01)

In the group with serious haemolytic disease (n 10) highly significant negative correlation with fraction B was observed ($r =$ 0.89 $0.001 >$ p) significant negative correlation to total protein content (0.81 $0.01 > p > 0.001$) and probably significant negative correlations to fractions A and C ($r =$ 0.73 and -0.67 $0.05 > p >$ 0.01)

No significant correlation could be found between the values for fraction D and the values for ΔE_{450} in normal patients and in the two groups of patients with isoimmunization. In samples taken during the 36th–37th week ($n = 11$) from the patients with haemolytic disease no significant correlation appeared with the values for haemoglobin and bilirubin in cord blood.

In normal patients delivered within 7 days of sampling a probably significant correlation appeared with infant birth weight ($n = 14$ $r = 0.63$ $0.05 > p > 0.01$). No significant correlation was observed with the placental weight in the same group

All samples were stored frozen and this resulted in precipitation of uric acid in some samples of amniotic fluid. This was not observed until all experiments were completed. The above values of the amount of material in fraction D thus may be less accurate. This would tend to weaken any existing correlations with other factors

Correlations between the electrophoretic protein fractions and the total protein content

In early normal pregnancies all three main fractions showed a highly significant correlation with the total protein content ($0.001 > p$ $= 15$) The coefficient for fraction A was 0.86 for fraction B 0.80 and for fraction C 0.99

In the later phase of normal pregnancies ($n =$ 4) fraction A showed a probably significant correlation with the total protein content ($r = 0.43$ $0.05 > p > 0.01$) Fractions B and C showed highly significant correlations with the total protein content ($r = 0.79$ and 0.94 $0.001 > p$)

Samples from Rh-immunized pregnancies complicated by slight haemolytic disease ($n = 15$) had highly significant correlations between the values for fractions A, B C and the total protein content ($= 0.77$ 0.91 0.96, $0.001 > p$) In the ten samples from pregnancies with serious haemolytic disease only fractions B and C showed significant correlations with the total protein content ($= 0.80$ and 0.96 $0.01 > p > 0.001$)

Correlations between the protein fractions and cord blood haemoglobin values

Ten patients with haemolytic disease were sampled during the 36th–37th week No significant

correlation existed between the concentrations of the protein fractions and the haemoglobin values for these patients.

Correlations between the protein fractions and cord blood bilirubin

Bilirubin values from 11 affected pregnancies sampled in the 36th–37th week were available. No significant correlations were found.

Correlation between the protein fractions and ΔE_{450} in amniotic fluid

Estimations of ΔE_{450} were available for 13 normal patients in the 33rd–40th week. A probably significant correlation existed between the values for fractions B and C and the ΔE_{450} values ($r = 0.56$ and 0.60, $0.05 > p > 0.01$).

Samples from isoimmunized patients showed no significant correlation between the values for the protein fractions and ΔE_{450} values for any of the subgroups.

Correlations between the protein fractions and infant birth weight or placental weight

Fourteen of the normal patients were delivered within 7 days after sampling. No significant correlation was found between the protein concentrations and infant birth weight or placental weight for these pregnancies.

DISCUSSION

Investigation of amniotic fluid protein fractions have in recent years been centered mainly around immuno-chemical determinations especially of the immunoglobulins. The results have shown a great variability both of the individual values and between different investigators (2, 3, 4, 6, 19, 20, 21, 24). Any constant findings that are characteristic of amniotic fluid from patients with haemolytic disease do not seem to have transpired from these investigations.

The order and also the mobilities of the protein fractions in free zone electrophoresis are the same as in Tiselius moving boundary electrophoresis (16) and essentially the same as in paper electrophoresis (?7). One can therefore easily calculate that the peaks A and C in the amniotic fluid electropherogram have the same mobilities as γ globulin and albumin respectively in the serum electropherogram (Figs. 1 and 4), while peak B has a mobility corresponding to α and β globulins. The high peak D in front of the protein peaks was found to be composed mainly of uric acid. The negative ninhydrin reaction indicates the absence of amino acids. The slowly increasing size of this peak with advancing pregnancy is in accordance with previous determinations of uric acid in amniotic fluid (5).

In a previous study it was observed that samples of amniotic fluid from pregnant women with a fetus slightly affected by haemolytic disease had a high mean content of protein compared both with normal pregnancies and with pregnancies complicated by serious haemolytic disease (14). In the present study it was found that the albumin fraction (fraction C) showed the greatest relative size in samples with the highest total protein content and then a decrease towards the end of pregnancy when the total protein concentrations were low. The absolute values for the albumin fraction also showed the highest correlation to the total protein values in amniotic fluid of all the fractions studied. This indicates that albumin is the main determinant of the total protein concentration in amniotic fluid. The values for the relative albumin content observed in this study were somewhat lower than those found by previous investigators in the later phase of pregnancy. Electrophoretic determinations have given values in the range 52.0–68.6% (1, 8, 22, 23, 6). Immunodiffusion techniques have given values of 60.0–66.0% (2, 6, 19). Heron (8) found an increasing percentage of albumin with advancing pregnancy which is in contrast to the tendency observed in the present study. Most investigators working with electrophoresis, however, have subjected the amniotic fluid to dialysis and concentration prior to electrophoresis which may cause disturbing absorption and denaturation of proteins and loss of low molecular weight substances. Gradient polyacrylamide gel electrophoresis in the present study indicates that amniotic fluid is comparatively rich in low molecular proteins which could possibly be eliminated by dialysis and this might have influenced the protein patterns.

The present study demonstrates a rather poor connection between the concentrations of the protein fractions and the clinical status of the infants in pregnancies complicated by haemolytic disease.

The slowest protein fraction (A) showed the highest correlation to the cord blood haemoglobin and bilirubin concentrations but it did not reach a significant level. The rather poor correlation between amniotic fluid total protein values and the same parameters was observed in a previous study (14).

The albumin is considered to be the main carrier of bilirubin in amniotic fluid (18, 25). In accordance with this the present study showed a probably significant correlation between the albumin fraction (C) and the ΔE_{450} values in normal patients. This correlation was weaker in patients with haemolytic disease. Haemolytic disease thus seems to cause divergent alterations in the amniotic fluid content of bile pigments and proteins.

Although duration of pregnancy has a pronounced effect on the concentrations of certain protein fractions the weight of the infant does not seem to be correlated to the protein concentrations prior to birth in normal pregnancies. The concentration of uric acid, however, seems to be dependent on infant birth weight to some extent. The negative correlations between uric acid concentrations and the concentrations of total protein and some of the protein fractions may simply be due to opposite trends for the levels with advancing gestation.

As the number of cases in the present study is rather small and distributed over several weeks of pregnancy comparisons between the clinical groups were sometimes difficult. The regression analyses then perhaps gives a better picture of the trends in the clinical groups (Fig. 4). No real difference could be demonstrated between normal patients and patients with haemolytic disease without fetal anaemia. However, a more pronounced downward trend for all three protein fractions was observed in isoimmunized patients with fetal anaemia and the difference was significant for the slowest fraction (A). This could indicate rapidly decreasing amounts of immunoglobulin in this group. Previous investigations with electrophoresis do not seem to have demonstrated any significant difference in the content of γ globulins between normal pregnancies and pregnancies complicated by haemolytic disease (1, 8, 26). An increased amount of β-lipoproteins, IgA and IgD has been found by Urategui-Gomez et al. (24).

The present study offers no explanation for the rapid decrease in protein concentrations and the low levels of uric acid in the cases with serious haemolytic disease. An increase in fetal swallowing with rapid elimination of proteins and uric acid could be a possible explanation. In the present study the blood group distribution was different in the two groups of Rh-isoimmunized pregnancies with haemolytic disease. In the group without fetal anaemia seven infants belonged to blood group 0, four to group A, none to group AB and three to group B. In the group with manifest anemia the corresponding figures were two, three, three and none. This might indicate that genetic factors are of importance perhaps affecting membrane permeability.

The different trends of the protein concentrations in the two groups of patients with haemolytic disease make it tempting to try correction of the protein content of the amniotic fluid as a therapeutic measure in cases with serious fetal disease. Injection of albumin in the amniotic cavity would perhaps facilitate binding and elimination of bile pigments from the fetus. Further studies are, however, required regarding the effects of such measures before they have any clinical application.

ACKNOWLEDGEMENTS

For valuable technical assistance at various stages of the work the authors are indebted to Mrs Inga Blomqvist and Mr Sture Jerstedt. Financial support has been obtained from the Swedish Natural Science Research Council, the Wallenberg Foundation and the Faculty of Medicine, University of Uppsala.

REFERENCES

1. Abbas, T. M. & Tovey, J. E.: Proteins of the liquor amnii. Brit Med J 1: 476, 1960.
2. Cherry, S. H., Rosenfield, R. E. & Kochwa, S.: Mechanism of accumulation of amniotic fluid pigment in erythroblastotic fetuses. Am J Obst Gynec 106: 797, 1970.
3. Curl, C. W.: Immunoglobulin levels in amniotic fluid. Am J Obst Gynec 109: 408, 1971.
4. Derrington, M. M. & Soothill, J. F.: An immunochemical study of the proteins of amniotic fluid and maternal and foetal serum. J Obst & Gynec Brit Comm 68: 755, 1961.
5. Doran, T. A., Bjerre, S. & Porter, C. J.: Creatinine, uric acid and electrolytes in amniotic fluid. Am J Obst Gynec 106: 325, 1970.

2 732954

6. Fischbacher P H. & Quinlivan, W L. G.. Qualitative and quantitative analysis of the proteins in human amniotic fluid. Am J Obst Gynec *108* 1051 1970.
7 Henry R. J Sobel, C. & Kim, J A modified carbonate—phosphotungstate method for the determination or uric acid and comparison with the spectrophotometric uricase method. Am J Clin Path *3* 152, 1957
8 Heron, H. J The electrophoresis of proteins of amniotic fluid. J Obstet Gynaec Brit Comm *73* 91 1966.
9 Hjertén, S.. Zone electrophoresis in columns of agarose suspensions. J Chromatog *1* 510 1963
10. — Free zone electrophoresis. Chromatog Rev *9* 122, 1967
11 — Free zone electrophoresis, theory equipment and applications. *In* Methods of Biochemical Analysis (ed. D Glick) *18*, 55 1970.
12. Hjertén, S & Jerstedt, S.. To be published.
13 Jonasson, L. E. & Johansson, E. D B.. Progesterone levels in amniotic fluid and plasma from women II. Levels during pregnancies complicated by Rh-immunization or hepatosis gravidarum. Acta Obst Gynec Scand *50*, 345 1971
14 Jonasson, L.E. Total protein content in amniotic fluid from normal pregnancies and from pregnancies complicated by Rh-isoimmunization. Acta Obst Gynec Scand *51* 187 1972.
15 — The clinical value of amniotic fluid analysis in pregnancies complicated by Rh-isoimmunization or hepatosis gravidarum. Acta Obst Gynec Scand 1973 in press.
16 Longsworth, L. G.. *In* Electrophoresis, Theory Methods and Applications (ed. M Bier), p. 137 Academi Press, New York, 1959
17 Liley A. W Liquor amnii analysis in the management of the pregnancy complicated by rhesus sensitization. Am J Obst Gynec *8* 1359 1961
18 *Mandelbaum, B., LaCroix, G C & Robinson, A. R.* Determinations of fetal maturity by spectrophotometric analysis of amniotic fluid. Obst & Gynec *29* 471 1967
19 Mendenhall, H. W Serum protein concentrations in pregnancy II. Concentrations in cord serum and amniotic fluid. Am J Obst Gynec *106* 581 1970.
20. Muller Eberhard, U & Bashore R.. Assessment of Rh disease by ratio of bilirubin to albumin and hemopexin to albumin in amniotic fluid. New Engl J Med *28* 1163 1970.
21 Ruoslahti, E., Tallberg, T & Seppälä, M.. Origin of proteins in amniotic fluid. Nature *21* 841 1966.
22. Stander R. M McNutt, C. C., Barton, D M. & Werts, C. E.. Characterization of proteins of human amniotic fluid by means of paper electrophoresis. Am J Clin Path *42* 125 1964
23. Usategui-Gomez, M., Morgan, D. F & Toolan, H. W A comparative study of amniotic fluid, maternal sera and cord sera by disc electrophoresis. Proc Soc E p Biol Med *123* 547 1966.
24 Usategui-Gomez, M., Hopkins, M. S. & Fernandez De Castro, A.. Serum proteins in amniotic fluids in erythroblastosis fetalis. Obst & Gynec *36* 865 1970.
25 Watson, D Mackay E. V & Trevella, W Amniotic fluid analysis and foetal erythroblastosis. Clin Chim Acta *1* 500, 1963
26. Wild, A. E.. The association between protein and bilirubin in liquor amnii. Clin Sci *1* 221 1961
27 Wunderly Ch.. *In* Electrophoresis, Theory Methods and Applications (ed. M. Bier) p. 179 Academic Press, New York 1959

Submitted for publication Nov 29 1972

Lars-Erik Jonasson
Department of Obstetrics and Gynecology
Academic Hospital
750 14 Uppsala
Sweden

Acta Obstet Gynec Scand 52: 355–359 1973

FETAL CHROMOSOME ANALYSIS AFTER TRANSCERVICAL PLACENTAL BIOPSIES DURING EARLY PREGNANCY

S. Kullander and B. Sandahl

From the Department of Obstetrics and Gynecology (Head: Professor S. Kullander), Malmö Allmänna Sjukhus, Malmö and the Department of Embryology (Head: Professor B. Källén), University of Lund, Lund, Sweden

Abstract. With an endocervicoscope 5 mm in diameter, and a fibre-optic system, it was possible to biopsy the chorion through the cervical canal, during early human pregnancies from 8 to 20 weeks of duration. 39 patients who applied for legal abortion were examined. Growth in tissue culture, with sufficient number of mitotic figures for sexing and chromosomal analysis was obtained in 20 out of 41 tests after 2-3 weeks. The karyotypes from the biopsy specimens were found to be normal and agreed with respect to sex with the legally aborted fetuses. 19 ... were observed for between 7 and 43 days following the biopsy did not show any disturbance of the pregnancy during that time. The practical usefulness of this technique, which was also tried in two cases of imminent abortion, is discussed.

The possibilities for the diagnosis of fetal sex and fetal chromosomal, enzymatic, and metabolic disturbances were very much facilitated by the introduction and extended use of early amniocentesis. A great many studies on amniotic fluid and its cells collected during the second trimester by transabdominal amniocentesis (for references, see (3, 4, 9)) have been published.

The method of examination of the amniotic fluid and cells, however, has certain limitations. Abdominal amniocentesis cannot be done without considerable technical difficulties and risks until the 14th week. 16 weeks gestation is said to be the ideal time. It is also questionable whether enough exfoliated cells are present in the amniotic fluid before this time (11). Nuclear sexing can be carried out cytologically on the cells in the amniotic fluid examined directly, but it is sometimes technically difficult. The reliability of the sexing method increases after culture of the cells, when a karyotype analysis may also be added. Metabolic disorders are best detected by studying cultivated amniotic fluid cells. Unfortunately, culture for chromosome studies has not been effective in all centres. The results so far fall short of the ideal of 100 % culture success rate and are said by different investigators to vary by as much as 23 to almost 100 %; at least, they appear to be less satisfactory than those achieved with skin cultures (10).

Another important problem is the length of time required to obtain a sufficient number of cultivated cells for adequate cytological evaluation. This time is given as up to 40 days, but most frequently it takes 3 weeks. The advanced time of gestation necessary for amniocentesis, plus a cell culture time of 3–6 weeks, means that the termination of a pregnancy when a defective fetus has been diagnosed will take place at a rather advanced stage, 17–20th weeks, which is medically unsatisfactory. Sampling and examining fetal cells for genetic diagnosis should preferably be done at a stage when if necessary a legal abortion could be performed no later than the 12th week, when a simple vacuum termination is possible.

A different technique for collecting cells from the conceptus for many types of morphological, biochemical, and haematological analysis, in direct or indirect form, is the transcervical placental biopsy. This requires a good and neat biopsy instrument. It is a fundamental fact that the placenta and the fetal membranes reflect the chromosomal and genetic constitution of the fetus; thus the sampling of chorionic villi may reveal the constitution of the fetus itself. Furthermore certain genetic defects in special fetal tissues, such

6. Fischbacher P H. & Quinlivan, W L G Qualitative and quantitative analysis of the proteins in human amniotic fluid. Am J Obst Gynec *108*. 1051 1970.
7 Henry R. J Sobel, C. & Kim, J A modified carbonate—phosphotungstate method for the determination or uric acid and comparison with the spectrophotometric uricase method. Am J Clin Path *8* 152, 1957
8 Heron, H. J The electrophoresis of proteins of amniotic fluid. J Obstet Gynaec Brit Comm *73* 91 1966.
9 Hjertén, S.. Zone electrophoresis in columns of agarose suspensions. J Chromatog *12* 510 1963
10. — Free zone electrophoresis. Chromatog Rev *9* 122, 1967
11 — Free zone electrophoresis, theory equipment and applications. *In* Methods of Biochemical Analysis (ed. D. Glick) *18* 55 1970.
12. Hjertén, S. & Jerstedt, S To be published.
13 Jonasson, L E. & Johansson, E. D B.. Progesterone levels in amniotic fluid and plasma from women II. Levels during pregnancies complicated by Rh-immunization or hepatosis gravidarum. Acta Obst Gynec Scand *50* 345 1971
14 Jonasson, L.E. Total protein content in amniotic fluid from normal pregnancies and from pregnancies complicated by Rh-isoimmunization. Acta Obst Gynec Scand *51*: 187 1972.
15 — The clinical value of amniotic fluid analysis in pregnancies complicated by Rh-isoimmunization or hepatosis gravidarum. Acta Obst Gynec Scand 1973 in press.
16 Longsworth, L. G *In* Electrophoresis, Theory Methods and Applications (ed. M Bier), p. 137 Academic Press, New York, 1959
17 Liley A. W Liquor amnii analysis in the management of the pregnancy complicated by rhesus sensitization. Am J Obst Gynec *82* 1359 1961
18 Mandelbaum, B LaCroix, G C. & Robinson, A. R. Determinations of fetal maturity by spectrophotometric analysis of amniotic fluid. Obst & Gynec *29* 471 1967
19 Mendenhall, H. W Serum protein concentrations in pregnancy II. Concentrations in cord serum and amniotic fluid. Am J Obst Gynec *106* 581 1970.
20 Muller-Eberhard, U & Bashore R.. Assessment of Rh disease by ratio of bilirubin to albumin and hemopexin to albumin in amniotic fluid. New Engl J Med *28* 1163, 1970.
21 Ruoslahti, E., Tallberg, T & Sipplli, M Origin of proteins in amniotic fluid. Nature *212* 841 1966.
22. Stander R. M McNutt, C. C., Barton, D M & Werts, C. E. Characterization of proteins of human amniotic fluid by means of paper electrophoresis. Am J Clin Path *4* 125 1964
23 Usategui-Gomez, M., Morgan, D F & Toolan, H. W A comparative study of amniotic fluid, maternal sera and cord sera by disc electrophoresis. Proc Soc Exp Biol Med *123* 547 1966.
24 Usategui-Gomez, M Hopkins, M S. & Fernandez De Castro, A. Serum proteins in amniotic fluids in erythroblastosis fetalis. Obst & Gynec *36*. 865 1970.
25 Watson, D MacKay E. V & Trevella, W Amniotic fluid analysis and foetal erythroblastosis. Clin Chim Acta *12* 500, 1965
26 Wild, A. E. The association between protein and bilirubin in liquor amnii. Clin Sci *1* 221 1961
27 Wunderly Ch.. *1* Electrophoresis, Theory Methods and Applications (ed. M. Bier), p 179 Academic Press, New York, 1959

Submitted for publication Nov 29 1972

Lars-Erik Jonasson
Department of Obstetrics and Gynecology
Academic Hospital
750 14 Uppsala
Sweden

Table II. *Interval between biopsy and legal abortion* (not including the two infected cases)

	<3 days	3–6 days	7–10 days	11–18 days	>18 days
No. of cases	13	3	12	2	5

had been given. In another case a multipara in the 18th week of pregnancy had a successful biopsy. She had a slight vaginal discharge at the time and culture for Neisseria was made from the cervix but accidentally destroyed in the laboratory. Tissue culture showed a male fetus. The patient developed fever at home, therefore a week later she was again admitted to the hospital and treated with antibiotics. A rising titre of gonococcal complement in the serum was then observed. A 24 cm long macerated male fetus was evacuated from the uterus by suction curettage. Recovery was uneventful.

Nothing abnormal was observed in the other cases at the legal abortion operations. Placental and fetal tissue was submitted to both routine histopathological examination and also special fetal dissection for determination of fetal sex. Tissue culture from the fetus was made to confirm karyotype and sex originally found in the biopsy specimen.

No bleeding, pains, or other signs of threatened abortion or fever were observed in any except the two cases mentioned above and the pregnancies were observed for up to a maximum of 43 days after the biopsy (Table II).

A high percentage of specimens, 19 out of 39 trials, were successfully cultured (Table I). The tissue pieces obtained must be inspected macroscopically once it has been taken. Villi in most cases can be directly observed. In a few instances the first grasp gave endocervical mucus only which was probably pushed up with the instrument to the lower pole of the pregnancy. Fig. 1 shows the typical microscopical picture of a biopsy specimen.

Finally the method was also used in two cases of imminent abortion to rule out chromosomal disturbances in the conception product. 1) A multipara had a biopsy taken in the 19th gestational week when treated in the ward for pains and bleeding. Culture showed a normal male karyotype. Much fibrin in the placenta was seen at microscopy of half of the biopsy. 14 days later a male fetus, 27 cm long, was spontaneously aborted. 2) A 20-year old woman, who had delivered one normal child previously, had a biopsy taken in the 12th gestational week when treated in the ward for signs of threatened abortion with pains and bleeding. No anaesthetic was used, and the lower pole of the pregnancy sac seemed normal at inspection. The pregnancy continued uneventfully and a normal healthy baby was born at term. No growth, however, was obtained in the cultured tissue obtained at biopsy.

The biopsy specimens were cut into small pieces and incubated without culture medium at 37 C for one hour. The medium was then added, this consists of 70% Parker solution, 20% human serum, 10% chicken embryo extract, and antibiotics. The culture medium was changed about one week later and changed a second time after a further week.

The cells were treated in a 0.25% solution of trypsin for one hour three days after the last change of medium. Treatment with colcemid for four hours followed, then fixing in acid for 2–3 hours and staining in 2% acetic acid-orcein. Cells were squashed in the usual manner. In 25 cells, the chromosome number was counted and the morphology evaluated. Four cells were photographed, and karyotypes were made.

Cytological findings

39 specimens were cultured. Of these, 20 grew. Twelve were male, seven were female, all of normal karyotype. In one case, two pieces of tissue were taken. One specimen showed female sex and the other male. Tissue culture of the aborted fetus showed male sex. Probably one biopsy was taken of maternal tissue and the other of fetal tissue. Most of the other 19 specimens which were not successfully cultived were infected by bacteria, and in some cases, even by flagellates. Probably the tissue became infected at the time of biopsy.

In 15 cases, the cytological findings following biopsy were correlated with the sex of the fetuses, which were later aborted. The fetuses were sexed either by macroscopic analyses or by tissue culture. In all cases, there was a complete correlation between the sex following biopsy and the sex of the aborted fetus (Table III).

Table I *Placental biopsies parity gestational week and successful culture*

	8–10	11 12	13–14	15–20	Total
0-parae	5	7	4	3	19
Multiparae	7	6	2	5	20
Total	12	13	6	8	39
Successful cultured	8	4	2	5	19

as phenylketonuria and haemoglobinopathies, cannot at present be diagnosed on amniotic cells, but this problem could probably be solved with the help of successful chorionic biopsies. Ultrastructural and histochemical observations could be made

A transcervical biopsy of chorionic tissue at about the two-month stage and a successful and rapid culture of the cells obtained would seem to lead to the solution of several problems. Indeed this was suggested and tried in 8 cases (6) In a further small series of 12 ambulant cases (7) spontaneous abortion occurred within a week in half of them They took their biopsy specimens with an instrument composed of a tube 6 mm in diameter and made their experiments on women who had permission for legal abortion. In primigravidae the cervical canal was found to be far too narrow for the instrument, being suitable only for multiparae. The pregnancies were usually terminated immediately after biopsy They could perform biopsy down to the 7th week of pregnancy but the 10th week was preferable. They got good growth in tissue culture but were unsuccessful with a fast cell culture method. Instead they had to use the traditional slower procedure with subculture of the cells which involved a culture time of 4–6 weeks and a fetal diagnosis at about the 15th week. In the tissue obtained from the chorionic biopsies, they reported mitotic figures good enough for chromosomal analysis in only 12 out of 34 cases. In the rest the growth was insufficient.

We now have used the transcervical approach and a new improved, and neat instrument in a large series of women in the early stages of pregnancy who required legal abortions, and have been able to watch the response to the biopsies for up to 6 weeks before the pregnancies were terminated

MATERIAL AND METHODS

For the inspection of the lower pole of the pregnancy sac and biopsy from the chorion an endocervicoscope designed by Menken (The Storz Company Tuttlingen, West Germany) with cold fibre-optic light was used. The diameter of this instrument is 5 mm, and a biopsy forceps is combined with the Hopkins 4 mm telescope.

The study was made on 39 cases of pregnancies requiring legal abortions for psycho-social reasons. The patients ages ranged from 16 to 43 years. Table I list data on their parity and the gestational age at the time of the procedure After two cases of infection were observed, sulfamoxol tablets were given in a dose of 0.5 g take daily fo 3–4 days before biopsy and after culturing for Neisseria, and then usually until legal abortion. The patient was placed in a dorsal lithotomy position. Some of the first cases were anaesthetized with a intravenous injection of a barbiturate followed by inhalation of halothane in a nitrous-oxygen mixture Later in the investigation, anaesthesia proved unnecessary The anterior lip of the cervix was grasped with a tenaculum. In some cases, the cervix was then gently dilated to Hegar 6, but usually the cervicoscope could be introduced easily through the internal cervical os without dilatation Biopsy is made under direct vision and small piece about 1 cubic mm, of the chorion was easily taken. Bleeding was minimal. The technique is quite simple and provides clear direct view of the lower pole of the pregnancy sac. The colour of the amniotic fluid can be seen, and sometimes also the fetus through the membranes.

The biopsy tissue was put into sterile Parker s sol and then used for tissue culture Half of the tiss some of the early cases was used for histological exa tion and was therefore f xed i 4% f rmalin, sectı after paraffin block ng in 5 μ sections, and stained haematoxylin-eosin.

The patients were usually observed f r one day in the hospital after biopsy but on xamination day after nev r showed any bleeding. Later n th vestigation, they were sent home nd legal abortions generally performed after one t three weeks.

RESULTS

Two patients had complications from the pr dure due to infection A l year-old nullip seeking legal abortion on social grounds, ha successful biopsy in the 12th week of gestat culture for Neisseria being made at the same ti She did not complain of anything abnormal (an innocent looking vaginal discharge being s The lower pole of the pregnancy sac seemed r mal in the cervicoscope Culture was succes and showed a normal male karyotype. Afte week at home she entered the hospital w fever and spontaneously aborted a male fe The culture result arrived and was positive Neisser Healing was uneventful after antibiot

Table II *Interval between biopsy and legal abortion not including the two infected cases)*

	<3 days	3–6 days	7–10 days	11 18 days	>18 days
No. of cases	13	5	12	2	5

had been given. In another case, a multipara in the 18th week of pregnancy had a successful biopsy She had a slight vaginal discharge at the time and culture for Neisseria was made from the cervix but accidentally destroyed in the laboratory Tissue culture showed a male fetus The patient developed fever t home: therefore a week later she was again admitted to the hospital and treated with antibiotics A rising titre of gonococcal complement in the serum was then observed A 24 cm long macerated male fetus was evacuated from the uterus by suction curettage. Recovery was uneventful.

Nothing abnormal was observed in the other cases at the legal abortion operations. Placental and fetal tissue was submitted to both routine histopathological examination and also special fetal dissection for determination of fetal sex. Tissue culture from the fetus was made to confirm karyotype and sex originally found in the biopsy specimen

No bleeding, pains, or other signs of threatened abortion or fever were observed in any except the two cases mentioned above and the pregnancies were observed for up to a maximum of 43 days after the biopsy (Table II).

A high percentage of specimens, 19 out of 39 trials, re successfully cultured (T ble I) The tissue piece obtained must be inspected macroscopically once it has been taken Villi in most cases can be directly observed In few instances the first grasp ga endocervical mucus only hich was probably pushed up with the instrument to the lower pole of the pregnancy Fig 1 show the typical microscopical picture of a biopsy specimen

Finally the method was lso used in t o cases of imminent bortion to rule out chromosomal disturbances in the conception product 1) A n IIpara had a biopsy taken in the 19th gestational week hen treated in the ward for pains and bleeding Culture showed normal male karyotype. Much fibrin in the placenta was seen at microscopy of half of the biopsy 14 days later a male fetus, 27 cm long, was spontaneously aborted. 2) A 20-year old woman, who had delivered one normal child previously had a biopsy taken in the 12th gestational week when treated in the ward for signs of threatened abortion with pains and bleeding. No anaesthetic was used, and the lower pole of the pregnancy sac seemed normal at inspection The pregnancy continued uneventfully and a normal healthy baby was born at term. No growth however was obtained in the cultured tissue obtained at biopsy

The biopsy specimens were cut into small pieces and incubated without culture medium at 37 C for one hour The medium was then added this consists of 70% Parker solution, 20% human serum, 10% chicken embryo extract, and antibiotics The culture medium was changed about one week later and changed a second time after a further week

The cells were treated in a 0.25% solution of trypsin for one hour three days after the last change of medium Treatment with colcemid for four hours followed then fixing in acid for 2–3 hours and staining in 2% acetic acid-orcein Cells were squashed in the usual manner In 25 cells, the chromosome number was counted and the morphology evaluated Four cells were photographed, and karyotypes were made

Cytological findings

39 specimens were cultured Of these 20 grew Twelve were male, seven were female ll of normal karyotype In one case two pieces of tissue were taken One specimen showed female sex and the other male Tissue culture of the borted fetus showed male sex Probably one biopsy was taken of maternal tissue and the other of fetal tissue Most of the other 19 specimens which were not successfully cultived were infected by bacteria, and in some cases, even by flagellates Probably the tissue became infected at the time of biopsy

I 15 cases, the cytological findings f flowing biopsy were correlated with the sex of the fetuses, which were later aborted The fetuses were sexed either by macroscopic analyses or by tissue culture. In all cases, there was complete correlation between the se following biopsy and the sex of the borted fetus (Table III)



Fig 1 Placental biopsy from lower egg-pole in the 13th gestational week in a nulligravidae. Half of the biopsy was successfully cultured and showed a normal female karyotype At following legal abortion, a female fetus was found. ×105 5 μ Htx-eosin.

DISCUSSION

The safety of any new technique used in clinical practice must be verified. Amniocentesis mostly transabdominal has been in use since about 1956, but only few records of harm to the mother or child are described (2, 5) The transvaginal route on the other hand carries an appreciable risk of spontaneous abortion (4) Foreign bodies, IUCD may be present in the uterus throughout the whole pregnancy without harming the embryo Then they are often found embedded in the membranes at the time of delivery

A possibility at both amniocentesis and placental biopsy is that the embryo may bleed into the mother so that she is sensitized against its blood This risk could be evaluated by examining the mothers blood for fetal erythrocytes after the biopsy The membranes could rupture at biopsy causing later spontaneous abortion. We did not observe this. Infection and septic abortion is a possibility that could be reduced to a minimum by vaginal-cervical culture and antibiotics before the procedure. Before we instituted this routine, we had two cases in our series where untreated gonorrhoea was present (with certainty in one case and strongly suspected in the other). Bleeding from the biopsy site might occur and harm the child. We never observed this side effect. An antifibrinolytic agent to the mother could probably minimize the blood loss (8). Finally there is a risk of abnormalities in the normal development of the child, produced by the diagnostic procedure and this must be weighed against the risks inherent in the genetic situation as the test will be limited to high risk cases in its practical use The value in genetic counselling is obvious, where there is a known high risk of a fetus affected by a chromosome anomaly or an inborn error of metabolism. Many parents will be willing to plan further children if offered such a test followed by the offer of a termination of the pregnancy if the embryo is shown to be affected. The potential value as a screening procedure for chromosome abnormalities also seems high in threatened abortion—and this was the indication for two tests in the present work—and in mothers more than 40 years of age Safety and reliability of the procedure must be established in larger series including the types of case described above.

The villous chorionic membrane in the 9th–10th week of pregnancy is free from the amnion and then offers good opportunity for transcervical

Table III

	Female	Male
Biopsy sexed by tissue culture	7 2	12[a] 6
Fetus sexed by macroscopic findings	5	7

[a] The case with two biopsies of different sex excluded

biopsy using the endocervicoscope. At the same time it is possible to inspect the amniotic sac and its fluid and sometimes also the fetus. This might provide valuable information in cases of suspected mole or fetal death.

At a more advanced stage of pregnancy placental biopsies can be made by transabdominal puncture and a few such cases have been reported (1).

The transcervical chorionic biopsy seems to offer several advantages over the traditional amniocentesis, but should still be regarded as potentially hazardous to the fetus. Errors can be made when the number of fetuses is incorrectly estimated and a biopsy taken from only one of two dizygotic placentas. Passage of fetal blood into the maternal blood-vessels is a possibility that should be borne in mind. Documents for termination could well, from an ethical point of view be arranged in many cases before the biopsy on the understanding that legal abortion should be done if the result is not technically successful and does not offer a good prognosis. The obstetrical department should be backed by a chromosome laboratory competent in this field. The indications for biopsy on intact pregnancies are still so rare that these, in the beginning, should be concentrated in a few places where they could benefit from the practice obtained. A thorough examination of either the fetus or the newborn is a prerequisite for establishing further the validity of the method.

REFERENCES

1. Alvarez, H. Amer J Obstet Gynec 95 538, 1966.
2. Cederbaum, S. D. Holzman, G. B. & Sparkes, R. S. Lancet 2 429 1971
3. Dorfman, A. (ed.) Antenatal diagnosis. The University of Chicago Press, Chicago, 1972.
4. Pocha, F. Clin Obstet Gynec 9 449 1966.
5. Gerbie A. B., Nadler H. L. & Gerbie, M. V. Amer J Obstet Gynec 109 765 1971
6. Hahnemann, N. & Mohr J. Bulletin of the European Society of Human Genetics 2 23, 1968
7. Hahnemann, N. & Mohr J. Bulletin of the European Society of Human Genetics 3 47 1969
8. Kullander, S. & Nilsson, L.M. Acta Obstet Gynec Scand 49 241 1970.
9. Nadler H. L. J Pediat 74 132, 1969
10. Nadler, H. L. & Gerbie, A. Obstet Gynec 38 789 1971.
11. Papp, Z., Gardo, S. Herpay G. & Arvay A. Obstet Gynec 36 429 1970.

Submitted for publication Nov 30, 1972

Stig Kullander
Department of Obstetrics and Gynaecology
Malmö Allmänna Sjukhus
Malmö
Sweden

Acta Obstet Gynec Scand 52 361–365 1973

SERUM LEVELS OF FSH AND LH FOLLOWING NORMAL PARTURITION

Sami A. H. Said and Leif Wide

From the Department of Obstetrics and Gynecology (Head: Prof. Carl Gemzell), and the Department of Clinical Chemistry (Head: Prof. Anders Ohlsson), University Hospital, Uppsala, Sweden

Abstract. Follicle-stimulating (FSH) and luteinizing hormones (LH) were measured by radioimmunosorbent assay in 133 serum samples after parturition from 10 normal healthy women. All cases were investigated at weekly intervals till resumption of menstruation and during the first menstrual cycle. The postpartum period, defined as the time between delivery and resumption of menstruation, was divided into lactation, partial lactation and non-lactation periods. Low or non-detectable FSH values were found during the first 2 weeks post partum. Human chorionic gonadotropin (HCG) was detected in the samples obtained during the first 2 weeks after delivery. During the remaining period of sampling, both FSH and LH concentrations were within the range found in the normal menstrual cycle excluding the midcycle peak. No LH values in the range of the midcycle peak could be detected in any of the samples obtained during the three periods. The average serum FSH value during lactation was significantly lower than that found during the period of non-lactation. This significant difference was attributed to the low FSH levels found during the first weeks after delivery. During non-lactation the mean serum FSH concentration was significantly higher than that of the luteal phase but similar to that of the follicular phase of the ovulatory control cycles.

Serum levels of FSH and LH during the normal human menstrual cycle and pregnancy have been extensively studied with various radioimmunoassay techniques but comparatively little has been reported on the serum concentrations of these pituitary gonadotropic hormones during the normal post-partum period. During the sixth post-partum week Jaffe et al. (3) found serum FSH and LH levels within the range of the normal menstrual cycle in both lactating and non-lactating women. Fuchs et al. (4) reported measurable plasma FSH and LH levels in a group of normal puerperal females with no striking differences between those who lactated and those who did not lactate. On the other hand Crystle et al. (2) found a significant difference in the concentration of FSH between lactating and non-lactating mothers. In the lactating women FSH values were lower than in those who were not lactating.

This contradiction warrants further studies to determine the normal hormone levels during the post-partum period. This would facilitate the diagnosis and treatment of post-partum endocrinopathies.

The present report deals with the measurement of both FSH and LH in the sera of healthy women investigated at weekly intervals during the post-partum period by a radioimmunosorbent technique.

MATERIAL AND METHODS

Ten normal females from the post-natal wards of the University Hospital of Uppsala, Sweden, were selected for the investigation.

The criteria of normality were:

1. Normal menstrual history before pregnancy.
2. Uneventful course of pregnancy terminating in a full-term delivery of a normal baby.
3. Routine medical examination and laboratory investigations proved normal.

All cases were studied from the first week after delivery. Five of the females were primiparae and 5 had had their second babies. The mean age of the group studied was 26.1 years with a range from 22 to 34 years. None of the women received any medication during the period of investigation. The duration of lactation was limited by the amount of milk production in each case. Addition of artificial feeds was decided upon, however, maternal milk was not enough for the baby. Accordingly the period elapsing between parturition and resumption of menstruation was divided into three periods:

1. Lactation. This period began immediately after delivery and ended with the addition of the first artificial feed.
2. Partial lactation. This period ranged from the addition of the first artificial feed to complete artificial feeding.
3. Non-lactation. This period began with complete arti-

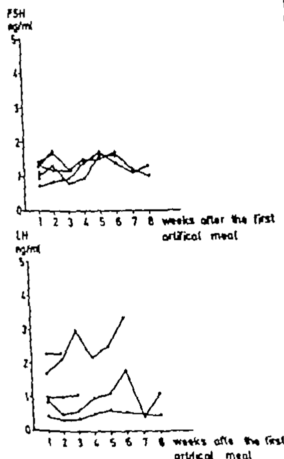


Fig. 2. Serum FSH and LH concentrations during partial lactation. ●, single values; ●—●, more than one value from the same patient.

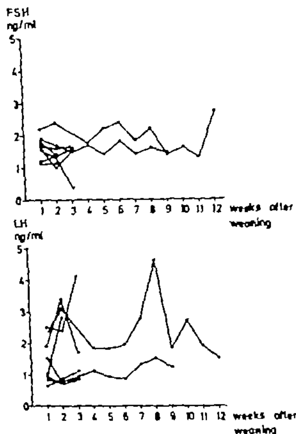


Fig. 3. Serum FSH and LH concentrations during non lactation. single values; ●—●, more than one value from the same patient.

etween the different periods was then made by sing the form Ia for Student's *t*-statistics on orrelated means. It was found that, for LH the alues during non-lactation were significantly igher (p 0.05) tha those found during partial lactation. No statistically significant differences were found between other groups except between the follicular and luteal phases of the control cycles ($p < 0.01$). For FSH the values during non lactation were significantly higher than during lactation ($p < 0.01$). The alues obtained during the first 2 weeks post partum were highly significantly lower ($p < 0.001$) than during non-lactation and also lower ($p < 0.05$) than during partial

Table I. *Mean FSH and LH during lactation, partial lactation, non-lactation and the control cycle*

	Lactation	Partial lactation	Non-lactation	Control cycle	Follicular phase	Luteal phase
FSH ng/ml Mean SD	0.91 0.48 (10)	1.28 0.22 (8)	1.47 0.29 (8)	1.19 0.34 (10)	1.31 0.18 (7)	1.09 0.2 (7)
LH ng/ml Mean SD	1.13 0.44 (5)	1.23 0.73 (8)	1.68 0.82 (8)	1.33 0.35 (10)	1.55 0.59 (7)	1.2 0.45 (7)

Figures in parentheses indicate number of women.
one mid-cycle peak FSH nd LH alue excluded

ficial feeding and ended with resumption of menstruation.

Blood samples were obtained from each female beginning during the first week after parturition. Samples were then obtained at weekly intervals except during the second and the third weeks post partum where samples were missing in five cases. A total of 93 samples were collected prior to the first menstruation. Blood samples were also collected during the first menstrual cycle and these were used for comparison of the hormone levels during the post-partum period. About 5 ml of antecubital venous blood was collected at each visit in the afternoon, i.e. between two meals during the period of lactation. Blood was left to clot at room temperature. The serum was then separated by centrifugation and kept at −20°C until assayed.

Immunoreactive LH and FSH in serum were assayed by a radio-immunosorbent technique (1., 13). LH in serum was measured by utilizing human pituitary LH (9) labelled with ^{125}I and rabbit anti-human pituitary LH. The LH preparation had a biological activity of 14 000 IU (2nd IRP HMG) per mg. The results were expressed in ng/ml. One ng of LH was equivalent to 84 ng of LER 907 in the immunoassay.

FSH in serum was measured by utilizing human pituitary FSH (9) labelled with ^{125}I and guinea-pig anti-human pituitary FSH. The FSH preparation had a biological activity of 12 000 IU (2nd IRP HMG) per mg. The results were expressed in ng/ml. One ng FSH was equivalent to 369 ng of LER 907 in the immunoassay.

Detection of HCG in the serum was performed by using two different radioimmunoassay systems, one for HCG and one for LH and calculation of the index of discrimination as described by Wide (14).

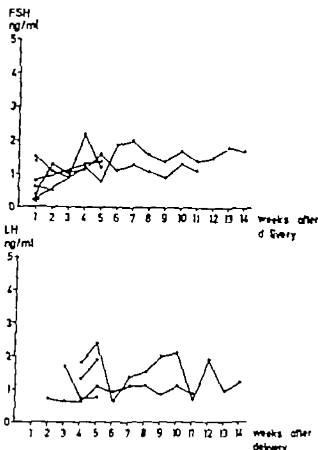


Fig. 1. Serum FSH and LH concentrations during lactation. ● single values, ●—● more than one value from the same patient.

RESULTS

FSH and LH concentrations in sera obtained from the 10 normal puerperal women during the three periods are presented in Figs. 1–3. The periods of lactation, partial lactation and non-lactation ranged between 1–14, 0–8 and 0–13 weeks, respectively. Menstruation was resumed in 2 cases immediately after stopping lactation. In 2 other cases the period of partial lactation was less than 1 week.

All samples during the first week and one sample during the second week after parturition were found to contain HCG. Pituitary LH could not be accurately estimated in these samples. Thereafter, during the period of lactation LH concentrations were in the range found in the normal menstrual cycle excluding the midcycle peak. Serum FSH levels were generally low during the first 2 weeks post partum. In 5 cases during the first week after delivery FSH values were lower than that of the normal follicular phase levels. After the first 2 weeks and during the rest of the lactation period FSH levels were similar to those found during the follicular phase of the normal menstrual cycle (Fig. 1).

During the period of partial lactation the concentrations of both LH and FSH fluctuated within the range of the normal menstrual cycle excluding the midcycle peak (Fig. 2).

During non-lactation the hormonal pattern was the same as that found during partial lactation. However, LH values just above the follicular phase levels of the normal menstrual cycle were found in 2 cases (Fig. 3).

No values in the range of the midcycle peak for either LH or FSH were encountered in any of the samples analysed during the three periods. The mean FSH and LH concentrations in the three periods and the control cycle together with the follicular and luteal phases of the control cycles which were ovulatory are presented in Table I. The mean values were calculated for each woman during the six periods presented in the table. The statistical analysis of differences

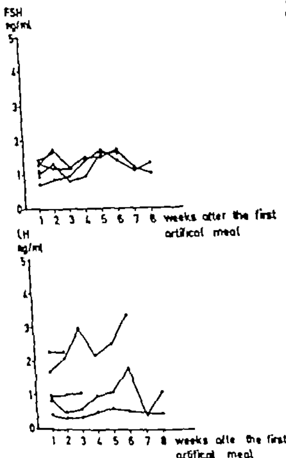


Fig. 2. Serum FSH and LH concentrations during partial lactation. ●, single values; ●—●, more than one value from the same patient.

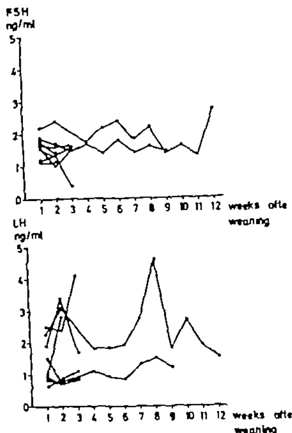


Fig. 3. Serum FSH and LH concentrations during non lactation. ●, single values; ●—●, more than one value from the same patient.

between the different periods was then made by using the formula for Student *t*-statistics on correlated means. It was found that, for LH, the values during non-lactation were significantly higher ($p < 0.05$) than those found during partial lactation. No statistically significant differences were found between other groups except between the follicular and luteal phases of the control cycles ($p < 0.01$). For FSH the values during non lactation were significantly higher than during lactation ($p < 0.01$). The values obtained during the first 2 weeks post partum were highly significantly lower ($p < 0.001$) than during non-lactation and also lower ($p < 0.05$) than during partial

Table I. *Mean FSH and LH during lactation, partial lactation, non-lactation and the control cycle*

	Lactation	Partial lactation	Non-lactation	Control cycle	Follicular phase	Luteal phase
FSH, ng/ml Mean ± S.D.	0.91 ± 0.44 (10)	1.28 ± 0.22 (8)	1.47 ± 0.29 (8)	1.19 ± 0.34 (10)	1.31 ± 0.18 (7)	1.09 ± 0.2 (7)
LH, ng/ml Mean ± S.D.	1.13 ± 0.44 (5)	1.23 ± 0.75 (8)	1.68 ± 0.82 (8)	1.33 ± 0.35 (10)	1.55 ± 0.59 (7)	1.2 ± 0.45 (7)

Figures in parentheses indicate number of women.
mid-cycle peak FSH and LH value excluded.

lactation. On the other hand comparison of FSH values obtained during lactation after the first 2 weeks to those during partial and non lactation showed no significant differences. The FSH values during non lactation were significantly higher than those found during the control cycle ($p < 0.05$) and the luteal phase ($p < 0.01$) but not significantly different from those of the follicular phase. Comparison between the other groups showed no statistically significant differences except between the follicular and luteal phases of the control cycles ($p < 0.01$)

DISCUSSION

Since the addition of the first artificial feed depended upon when the secretion of milk decreased and since the duration of lactation was limited by the amount of milk produced in each case the method used to divide the post-partum period was in accord with the normal sequence of events, i e lactation partial lactation and non lactation Moreover no medication was allowed during the investigation in order not to disturb the endocrine function of the pituitary gland.

In this study HCG was found in all the samples obtained during the first week post partum and in one sample during the second week. These results agree with those of Midgley & Jaffe (7) and Yen et al. (15) who reported that the high serum HCG levels encountered during pregnancy were not cleared until after 12 to 14 days post partum. Pituitary LH concentration could not be estimated accurately in the samples that contained HCG Cross reactivity between LH and HCG has been shown previously by Moudgal & Li (8) and by Wide et al. (11) After clearance of HCG LH values were found within the range of the normal menstrual cycle excluding the mid cycle peak.

Pituitary FSH production during pregnancy seems to be suppressed According to Midgley (6) no measurable levels of FSH were found in a group of pregnant women. The low pituitary FSH levels found in this study during the first 2 weeks after parturition might be attributed to the persisting effect of pregnancy This was in agreement with the report of Jaffe et al. (5) who found during pregnancy a decrease in the serum FSH that continued until 15 days after delivery

The concentrations of both LH and FSH after the first 2 post partum weeks until resumption of menstruation were similar to the levels found during the normal menstrual cycle excluding the midcycle peak. The finding of the significantly lower values of FSH during lactation than during non lactation was in agreement with that reported by Crystle et al. (2). However in the present study this significant difference was found to be due to the low FSH values during the first 2 weeks after delivery When these values were excluded no significant difference was found between the three periods. This, on the other hand, was in accord with the previous reports of Faiman et al. (3) and Fuchs et al (4) Nor did Jaffe et al. (5) find any change in the serum concentrations of either FSH or LH in a normal female followed daily between the 5th and the 74th day after delivery

It has been reported previously that after delivery there is a low incidence of ovulation before the first menstruation Sharman (10) concluded from studies on endometrial biopsies after normal parturition that only 25% of first menstruations were preceded by ovulation Cronin (1) found nearly the same percentage by recording the daily body temperature in a group of normal post partum females

In the present investigation when the different periods were compared with the two phases of the ovulatory control cycles it was found that FSH values during non-lactation were at levels consistent with the follicular phase and significantly higher than luteal phase levels This might indicate that the first menstruation was not preceded by a luteal phase in most of the cases. The absence of LH values in the range of the midcycle peak in any of the samples analysed might be due to the regime of sampling or to the fact that in most of the cases ovulation did not occur during the post partum period In fact, according to the serum progesterone levels estimated in the samples obtained before resumption of menstruation, seven cases were anovulatory two ovulatory and one indefinite

ACKNOWLEDGEMENT

We are indebted to Dr Paul Roos at the Institute of Biochemistry Uppsala, for supplying the highly purified LH and FSH preparations W acknowledge with grati-

take the help of our colleagues who assisted in selection of the cases for the study. The skilful technical assistance of Mr Christer Bengtsson is deeply appreciated. S. Said expresses his cordial thanks to Professor C. Gemzell for being received as Post-doctoral Fellow in his department, and extends his thanks to the Ford Foundation for the scholarship they offered to him. This study is supported by the Swedish Medical Research Council (grant no. 13-3145).

REFERENCES

1. Cronin, T. J. Influence of lactation upon ovulation. Lancet *II* 422, 1968.
2. Crystle, C. D. Powell, J. & Stevens, V. C. Plasma gonadotrophins during the postpartum period. Obstet Gynec 36 887 1970
3. Faiman, C., Ryan, R. G. Zwirek, S. J. & Rubin, M. E. Serum FSH and HCG during human pregnancy and puerperium. J Clin Endocr 28 1323 1968
4. Fuchs, F. Behrig, C. G. Frandsen, V. A., Jedinovich, J. B., Moller K. J. A., Saunders, D. M. & Saxena, B. B. Pituitary and ovarian hormone levels during lactation. Acta Endocr, Suppl *155* Abstract 40, 1971
5. Jaffe, R. B., Lee, P. A. & Midgley A. R., Serum gonadotrophins before, at the inception of and following human pregnancy J Clin Endocr 29 1281 1969
6. Midgley A. R. Radioimmunoassay for human FSH. J Clin Endocr 27 295 1967
7. Midgley A. R. & Jaffe, R. B. Regulation of human gonadotrophins. II. Disappearance of human HCG following delivery J Clin Endocr 28 1712, 1968.
8. Moudgal, N. R. & Li, C. H. An immunological study of human pituitary interstitial cell-stimulating hormone. Nature (London) *191* 192, 1961
9. Roos, P. Human follicle stimulation hormone. Acta Endocr Suppl *131* 1, 1968.
10. Sharman, A. The Reproductive Physiology of the Postpartum Period. 1st edition. Livingstone London, p. 70, 1966.
11. Wide, L., Roos, P. & Gemzell, C. Immunological determination of human pituitary LH. Acta Endocr 37 445 1961
12. Wide L. & Porath, J. Radioimmunoassay of proteins with the use of Sephadex-coupled antibodies. Biochim Biophys Acta *130* 257 1966.
13. Wide, L. Radioimmunosorbent assays employing immunosorbents. Acta Endocr Suppl. *142* 207 1969 *a*.
14. — Early diagnosis of pregnancy Lancet *II* 863 1969 *b*
15. Yen, S. S. C., Llerena, O. Little, B. & Pearson, O. H. Disappearance rates of endogenous luteinizing hormone and chorionic gonadotrophin in man. J Clin Endocr 28 1763, 1968.

Submitted for publication Dec. 5 1972

Samy Said
Department of Obstetrics and Gynaecology
University Hospital
S-750 14 Uppsala
Sweden

Acta Obstet Gynec Scand 52: 367–371 1973

TWIN PREGNANCY

The Value of Prophylactic Rest in Bed and the Risk Involved

Bent Laursen

From the Department of Gynaecology and Obstetrics (Heads: J. J. Jacobsen, K. Kristoffersen and G. Thomsen Pedersen), Odense Hospital, Odense, Denmark.

Abstract. A total of 315 twin pregnancies delivered during a 12 year period are reviewed, first to assess the value of admitting the mother to hospital during the last few weeks of pregnancy and secondly to analyse the perinatal mortality. Rest for the mother in hospital will prolong the pregnancy and increase the birth weight of the children significantly and will reduce the perinatal mortality. Some of the perinatal deaths occur in the neonatal period in a group of infants delivered before the end of the 35th week of gestation and, in almost all these cases, the cause of death is respiratory failure. If delivery takes place after the 35th week of gestation, perinatal death occurs mainly as intrauterine death. Possible causes and consequences are discussed.

The aim of the obstetrician always has been to examine and treat pregnant women with the object of diminishing the dangers connected with pregnancy, delivery and puerperium. Because of improved diagnostic and therapeutic facilities, maternal morbidity and mortality have been reduced significantly in recent years. Now our most important aim is to contribute to a reduction in perinatal mortality, and in this connection particular effort has been made to protect special high risk groups of pregnant women. Mothers carrying twins constitute such a group.

Several surveys have proved that the predominant cause of perinatal death in twins is a low birth weight and pathological conditions arising as a result of this. Both in single pregnancies and in twin deliveries, the most important prophylactic measures against infant mortality are those which might contribute to a reduction in the frequency of prematurity or, in another way, to help to increase the birth weight.

Since the beginning of the 1950s British authors in particular have emphasized that rest in bed, preferably during a stay in hospital, is such a prophylactic measure. Bartsch (1) published a survey of some of these studies. The usual recommendation is admission from about the 30th to about the 36th week of pregnancy.

During recent years, rest during a stay in hospital has been a fundamental principle in the care of mothers carrying twins in the Department of Obstetrics at the Odense Hospital.

MATERIAL

In order to take stock of these efforts, the case records of all twin pregnancies delivered in this Department throughout the 12 year period 1958–1970 are reviewed. Table I presents a survey of these 315 pregnancies. This material is divided into two groups, each of which covers 6 years. The division is made in 1964 because in that year an increase in the number of beds available permitted admission of pregnant women to this Department exclusively for the purpose of resting mothers in high risk groups. Hence, Group I comprises 127 patients who could be admitted to the Department only if serious pregnancy complications made such admission necessary. Group II includes 188 pregnant women who could all be admitted solely for rest as soon as their twin pregnancy was diagnosed.

The two groups are identical with regard to the age and parity of the women, the incidence of diseases, the nature and frequency of pregnancy complications, the complications during labour and most of the other usual criteria, and, in these respects, they do not differ from similar series collected in Western European countries during the same period.

RESULTS

Before prescribing a prolonged period of rest the diagnosis of twin pregnancy must be verified. Attempts to establish this diagnosis were made first

Table I *Number of twin deliveries 1958–70*

Year	No of deliveries	Total
1958	17	
1959	20	
1960–61	22	
1961–62	24	
1962–63	26	
1963–64	18	
Group I		127
1964–65	31	
1965–66	31	
1966–67	24	
1967–68	41	
1968–69	30	
1969–70	31	
Group II		188
Group I + II		315

by clinical examination. During the first part of the survey period a radiological examination of the abdomen was the only supplementary investigation available whereas, during the second period the diagnosis of twin pregnancy was made primarily with the aid of fetal electrocardiography or an examination for two fetal heart recordings with diagnostic ultra sound employing the Doppler principle. Only if a definite diagnosis was not obtained was an X-ray examination ordered.

The results of these diagnostic procedures are summarized in Table II. The significantly higher diagnostic accuracy rate in Group II ($p < 0.05$) firstly may be because the clinicians were more alert to the possibility of multiple pregnancy and secondly because repeated examinations are easier

Table II *Number of twin pregnancies diagnosed before onset of labour*

Group I	70 out of 127 pregnancies	55 %
Group II	126 out of 188 pregnancies	67

Table III *Duration of rest in hospital*

Pregnant women	No of cases
Admitted for more than 10 days	79
Admitted less than 10 days	19
Not admitted	28
With diagnosed twin pregnancy	126

Table IV *Distribution of 630 infants according to birth weight and gestational age within the various groups*

	Gestational age (weeks)					
	< 29	29–32	33–36	37–40	> 40	Total
Group I						
< 2 000 g	4	15	18	20	0	57
2 001–2 500 g	0	1	23	49	11	84
> 2 500 g	0	0	5	87	21	113
Group II not relieved						
< 2 000 g	4	19	32	10	0	65
2 001–2 500 g	0	1	32	4	3	40
> 2 500 g	0	0	10	72	11	93
Group II relieved						
< 2 000 g	0	0	7	5	1	13
2 001–2 500 g	0	0	11	29	0	40
> 2 500 g	0	0	2	92	11	105

with fetal electrocardiography and diagnostic ultra-sound.

To prevent low birth weight one or more daily intervals of prophylactic rest are advised, as soon as the diagnosis of twin pregnancy is established, and admission to the obstetrical ward is offered from the 28th or 30th week of pregnancy until the end of the 36th or 37th week, as far as the available number of beds in the ward permits.

However, not all mothers with confirmed twin pregnancies accepted the offer of admission for rest. Table III shows the number of cases in which the offer was accepted. Twenty-eight out of 126 women with twin pregnancy in Group II refused the offer either because they thought that sufficient rest could be obtained in their own homes, or because they could not possibly afford the time to stay in hospital. Nineteen mothers were admitted for periods of less than 10 days and consequently for shorter periods than are generally considered necessary to influence the course of the pregnancy. Hence only 79 mothers (42 % of the total number in Group II) stayed in the Department for a period long enough to be considered adequate. The average period of admission was 28 days.

The relationship between perinatal mortality and low birth weight which has been shown by several studies (1) provides a justification for an attempt to assess whether rest has had any demonstrable influence on these two variables.

Table IV shows the 630 infants in the series, distributed according to birth weight and gestational age within the various groups. Comparison of Group I with that part of Group II in which the infants were born to mothers who had not rested for 10 days or longer shows no statistically significant difference between the numbers in individual weight groups, and also the difference between the number of pregnancies of up to 252 days' duration (36 weeks) and the number of pregnancies lasting for 253 days or more, is *not significant*.

However, if the number of infants in Group II born to mothers who had rested for more than 10 days, is compared with the number of infants born to mothers who had an inadequate period of rest in the same group, there are, both in the weight group below 2 000 g (13 against 65 children) and in the weight group above 2 500 g (105 against 93 children), differences which, assessed by the chi²-test, are highly significant ($p < 0.001$) so that a pronounced relationship between rest and higher birth weight must be considered probable. Similarly the duration of pregnancy is found to be longer in mothers who had adequate period of rest in Group II, because 68 out of 79 pregnancies lasted up to the 253rd day or later, which is significantly more ($p < 0.05$) than the corresponding number in the part of Group II who had inadequate rest (60 out of 109).

The total perinatal mortality rates[1] are shown in Table V. The rate for the entire series was 81 per 1 000, and the difference between the rates contributed by Groups I and II as a whole is insignificant (p 0.4). On the other hand there is a significantly lower (p 0.001) mortality in the part of Group II who had adequate rest, compared with the remaining mothers in that group.

If the figures are corrected for infants having a birth weight < 1 000 g, the difference is somewhat reduced, as seen in Table VI, but the difference demonstrated in *Group II is, however, still* significant (p 0.05) and the perinatal mortality in pregnancies, during which the period of rest was inadequate, is still seen to be nearly three times as high as the corresponding value found in those babies of mothers who had adequate rest. The perinatal mortality in the latter group, however, is still several times that found in single pregnancies.

A survey of the causes of death in individual children did not produce in about 50 % of the cases, a satisfactory explanation of the death, in spite of the fact that attempts were made to elucidate the conditions by a retrospective critical review of the course of labour and autopsy of the dead infant.

By classifying the perinatal deaths in the whole series according to the relationship between stillbirths and neonatal deaths and the gestational age of the infant, it is possible to segregate two large groups. It appears from Table VII that out of 33 perinatal deaths where delivery occurred on the 245th day of pregnancy or earlier, 30 infants died *after* birth. Thirteen of these children weighed 1 000 g or less, in all the others the births weight was ≤ 2 500 g. The calculated average weight of all the 30 children was 1 315 g, S.D. 415 g. The corresponding average weight of the other twin was 1 340 g, S.D. 425 g. It should be emphasized that in 7 cases both the twins died, and the individual infants are then included in the calculation of the average weight of both. If a *t* test of the difference between the average weights is made, the resulting difference is found to be

Table V *The total perinatal mortality*

	No. of infants	‰
Group I	23 out of 254	90
Group II, total	28 out of 376	74
Not relieved	23 out of 218	104
Relieved	5 out of 158	32
Total	51 out of 630	81

Table VI *Perinatal mortality among infants* 1 000 g

	No. of infants	‰
Group I	16 out of 245	65
Group II, total	23 out of 371	61
Not relieved	18 out of 213	85
Relieved	5 out of 158	32
Total	39 out of 616	63

[1] No. [illegible] of stillborn infants 1 000 g plus liveborn infants dying 168 hours after birth per 1 000 stillbirths 1 000 g plus live births.

[illegible] perinatal [illegible] is selected because there [illegible] obvious [illegible] between neonatal death and stillbirth at the time.

1 [illegible]

Table I *Number of twin deliveries 1958–70*

Year	No of deliveries	Total
1958	17	
1959	20	
1960–61	22	
1961–62	24	
1962–63	26	
1963–64	18	
Group I		1 7
1964–65	31	
1965–66	31	
1966–67	24	
1967–68	41	
1968–69	30	
1969–70	31	
Group II		188
Group I + II		315

by clinical examination During the first part of the survey period a radiological examination of the abdomen was the only supplementary investigation available whereas, during the second period the diagnosis of twin pregnancy was made primarily with the aid of fetal electrocardiography or an examination for two fetal heart recordings with diagnostic ultra-sound employing the Doppler principle. Only if a definite diagnosis was not obtained was an X ray examination ordered

The results of these diagnostic procedures are summarized in Table II The significantly higher diagnostic accuracy rate in Group II ($p < 0.05$) firstly may be because the clinicians were more alert to the possibility of multiple pregnancy and secondly because repeated examinations are easier

Table II *Number of twin pregnancies diagnosed before onset of labour*

Group I	70 out of 127 pregnancies	55 %
Group II	126 out of 188 pregnancies	67

Table III *Duration of rest in hospital*

Pregna t women	No. of cases
Admitted for more than 10 day	79
Admitted less than 10 days	19
Not admitted	28
With diagnosed twi pregnancy	1 6

Table IV *Distribution of 630 infants according to birth weight and gestational age within the various groups*

	Gestational age (eeks)					
	<29 29–32		33–36	37–40	>40	Total
Group I						
<2 000 g	4	15	18	20	0	57
2 001 2 500 g	0	1	23	49	11	84
> 500 g	0	0	5	87	21	113
Group II not relieved						
< 000 g	4	19	3	10	0	65
001 500 g	0	1	32	4	3	60
> 500 g	0	0	10	77	11	93
Group II relieved						
<2 000 g	0	0	7	5	1	13
2 001 500 g	0	0	11	29	0	40
>2 500 g	0	0	2	92	11	105

with fetal electrocardiography and diagnostic ultra-sound.

To prevent low birth weight one or more daily intervals of prophylactic rest are advised as soon as the diagnosis of twin pregnancy is established, and admission to the obstetrical ward is offered from the 28th or 30th week of pregnancy until the end of the 36th or 37th week as far as the available number of beds in the ward permits.

However not all mothers with confirmed twin pregnancies accepted the offer of admission for rest Table III shows the number of cases in which the offer was accepted Twenty-eight out of 1 6 women with twin pregnancy in Group II refused the offer either because they thought that sufficient rest could be obtained in their own homes, or because they could not possibly afford the time to stay in hospital. Nineteen mothers were admitted for periods of less than 10 days and consequently for shorter periods than are generally considered necessary to influence the course of the pregnancy Hence only 79 mothers (4 [illegible] of the total number in Group II) stayed in the Department f r a period long enough to be considered adequate The a erage period of admission was 8 days

The relationship between perinatal mortality and low birth weight which has been shown by several studies (1) provides a justification for an attempt t a ses whether rest has had any demonstrable nfluence on these two variables

of such investigations are available, the present aim of the obstetrician must be the careful supervision of mothers with twins both after and before 35th week of pregnancy. Up till now the practice in this Department has been to discharge the mother between the 35th and 37th week of pregnancy and to see her weekly as an out-patient, as is done in single pregnancies, because the risk of premature delivery has passed. However, on the basis of the present study it appears that this danger is followed by the risk of intrauterine death, perhaps because of placental insufficiency. Therefore, the patient should not be discharged but offered continued rest in hospital, during which time tests of placental function are increased and, if signs of placental failure appear, labour should be induced.

REFERENCES

1. Bartsch, F. K.: Trillinggraviditet. *I*: Nya forskningar och rön, Obstetrik och Gynekologi 3 (ed. E. Brody & P. Bergman), p. 116. Akademiförlaget, Stockholm, 1946.
2. Kloosterman, G. J.: On intrauterine growth. Int J *Gynec Obstet 8*: 895, 1970.
3. Naaktgeboren, C. & Stegeman, J. H. J.: Untersuchungen über den Einfluss des Uterus und der Placenta auf das fetale Wachstum und das Geburtsgewicht mit besonderer Berücksichtigung des Schafes. Z Tierzücht Zücht Biol 85: 245, 1969 (cit. G. J. Kloosterman, 1970).

Submitted for publication Dec. 5, 1972

Bent Larsen
Sct. Jørgensgade 11
9000 Aalborg
Denmark

Table VII *Time of death related to time of birth and gestational age by birth*

Time of death	Died before birth	Died during birth	Died after birth
Birth on the 245th day or earlier	2	1	30
Birth on the 246th day or later	14	1	3

not significant ($p > 0.8$). The average weight of the placentae from the 30 pregnancies is found to be 715 g S.D 140 g.

In 37 pregnancies, not complicated by perinatal mortality where delivery occurred on the 245th day or earlier the average birth weight of twins A and B was 1 880 g S D 427 g and 1 874 g S.D 406 g, respectively These figures show no mutually significant difference, but they are both statistically higher than the average weight for children in the former group ($p < 0.01$) Also the average weight of the placentae was higher 825 g, S.D 169 g.

As would be expected in almost all of these cases, the cause of death was respiratory failure If this group is to be reduced it must be done primarily by the better diagnosis of twin pregnancy and the treatment of all the mothers by rest in hospital.

Another group of 14 infants born on the 246th day of pregnancy or later were all stillborn None of these infants had malformations or other organic changes demonstrable by autopsy which might explain intrauterine death. Five of the placentae had large or small infarctions, which might have played a role in the course of events, but in the remaining cases the causes of death must be considered unknown The average weight in the 14 stillborn infants was 2 040 g S.D 590 g while the other twin weighed on an average 2 700 g S D 575 g. In only one case did both twins die. The *t*-test of the difference between the average weights in this group showed that the difference was significant ($p < 0.01$) If a *t* test is made of the difference between the average weight of stillborn infants in this group and the neonatal deaths in the former group, there is also a significance at the 1% level The average placental weight was 810 g, S.D 85 g.

In 237 pregnancies, delivery of two live infants occurred after the 246th day of pregnancy The average birth weight of twins A and B was 2 708 g, S.D 487 g and 2 645 g, S.D 515 g, respectively These weights are both significantly higher than the average weights of the 14 stillborn twins mentioned above ($p < 0.01$). The average weight of the placentae of these 231 pregnancies was 1 06_ g, S.D 203 g which is significantly higher than the average placental weight in pregnancies, in which intrauterine death occurred before delivery on the 246th day or later

DISCUSSION

Hence the present series shows a statistically significant lower birth weight in children dying in utero before delivery on the 246th day of pregnancy or later and at the same time a significantly lower weight of the placentae in these pregnancies. This might indicate that placental insufficiency was responsible for these intrauterine deaths. Any confirmation of this assertion cannot be obtained from the present survey because only occasional tests of placental function were made during these pregnancies. In any case these findings agree with a theory advanced by Kloostermann (2) From a survey of 80 000 births and 30 000 placentae from two Dutch university clinics, by weighing children and placentae he found evidence that increase in weight and further development of the placenta ceases at a time between the 34th and the 36th week of pregnancy After that time the placenta is only able to meet increasing fetal demands because of its extra capacity and when this functional reserve is depleted, labour will generally commence In twin pregnancies the placental weight on average was 20% lower per infant and the placenta stopped growing about 1–2 weeks earlier than in single pregnancies. This theory was handsomely confirmed by the biologists Naaktgeboren & Stegeman (3) by experimental studies in pregnant sheep

Consequently it is considered that the cause of fetal death in the majority of the intrauterine deaths in the present series is placental insufficiency because the pregnancy was prolonged beyond the optimum gestational age However it would be necessary to carry out prospective studies including an assessment of placental function before this could be confirmed. Until the results

of such investigations are available, the present aim of the obstetrician must be the careful supervision of mothers with twins both after and before 35th week of pregnancy. Up till now the practice in this Department has been to discharge the mother between the 35th and 37th week of pregnancy and to see her weekly as an out-patient, as is done in single pregnancies, because the risk of premature delivery has passed. However, on the basis of the present study it appears that this danger is followed by the risk of intrauterine death, perhaps because of placental insufficiency. Therefore, the patient should not be discharged, but offered continued rest in hospital, during which time tests of placental function are increased and, if signs of placental failure appear, labour should be induced.

REFERENCES

1. Bartsch, P. K. Tvillinggraviditet. *In* Nya forskninger och rön, Obstetrik och Gynekologi 3 (ed. S. Brody & P. Bergman), p. 116. Akademiförlaget, Stockholm, 1966.
2. Kloosterman, G. J. On intrauterine growth. Int J Gynec Obstet 8 895 1970.
3. Naaktgeboren, C. & Stegeman, J. H. J. Untersuchungen über den Einfluss des Uterus und der Placenta auf das fetale Wachstum und das Geburtsgewicht mit besonderer Berücksichtigung des Schafes. Z Tierzucht Zucht Biol 85 245 1969 (cit. G. J. Kloosterman, 1970)

Submitted for publication Dec 5 1972

Bent Larsen
Sct. Jørgensgade 11
9000 Aalborg
Denmark

Acta Obstet Gynec Scand 52 373–379 1973

AMENORRHEA FOLLOWING USE OF COMBINED ORAL CONTRACEPTIVES

Mirjam Furuhjelm and Kjell Carlström

From the Department of Obstetrics and Gynecology (Head: Prof. A. Ingelman-Sundberg), Karolinska Institutet, Sabbatsberg Hospital, Stockholm, Sweden

Abstract. 249 cases of long-standing amenorrhea after the use of oral contraceptives have been examined clinically and their hormonal excretion in the urine has been determined in April 1972. Follow-up of the patients was undertaken and 178 had answered before May 1, 1972. 59.3% of the patients had recovered spontaneously: 38 of them had amenorrhea from 6 to 12 months and 69 more than 12 months. 72 patients still had amenorrhea at the time of the follow-up. Of the patients 122 probably had amenorrhea as a direct result of the treatment. In the other patients other factors could be taken into consideration as being the cause of amenorrhea. Three patients had premature menopause. In our series 35.4% of the patients had had menstrual irregularities antedating the use of oral contraceptives. The frequency of previous oligo-amenorrhea among post "pill" amenorrhea cases is obvious and such women would be better to choose another kind of contraception. The longest period of amenorrhea before treatment was 39 months. A low excretion of low polar oestrogens is associated with long duration of the amenorrhea. No treatment should be used in patients with moderate excretion of oestrogens in the urine except for those who desire to become pregnant. Treatment with bromocriptine or clomiphene and HCG is then the method of choice. Patients with low excretion of oestrogens in the urine can be treated with low doses of ethinyloestradiol (10–20 µg daily) in order to avoid atrophy of the genital organs and to stimulate the FSH and LH releasing factor. If they want children, HMG followed by HCG should be used as FSH/LH releasing hormones.

Two important centres in the hypothalamus are believed to be concerned in the control of pituitary function. A centre in the medial part of the hypothalamus is believed to be responsible for a constant basal production of gonadotropins maintaining basal ovarian activity. Another centre in the preoptic area of the anterior hypothalamus is thought to be responsible for the surges of gonadotropin release which lead to maturation of the follicle and to ovulation. Organic lesions and probably also suppression of the hypothalamus lead to disturbances at two levels of severity. In the milder form preoptic activity is abolished and surges of gonadotropins do not occur: basal production remains, however. Oestrogen production is at comparatively normal levels and menstruation may occur though cycles are of course anovulatory. In the more severe form, the centre governing basal production of gonadotropin is also involved. Gonadotropin and oestrogen production falls and complete amenorrhea develops.

From experiments on animals it is known that the administration of oestrogen and progesterone inhibits the neuronal activity of the hypothalamus. Administration of these substances to human beings diminishes the release of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) and thus inhibits ovulation. The excretion of FSH and LH in the urine is low and there is no ovulation peak. Oral contraceptive suppression of FSH and LH is maximal after three treatment cycles (28). This suppression is maintained while the patient continues to use the agent. Prolonged treatment with substances which diminish or inhibit the gonadotropin-releasing factors may also have a repressive effect on other releasing factors in the hypothalamus.

It was believed that after inhibition of hypothalamus a rebound effect follows, following cessation of the treatment. Instead several reports have drawn attention to the fact that menstrual disturbances and amenorrhea may follow the use of oral contraceptives (4, 10, 11, 13, 14, 16, 19, 1, 3, 4, 5, 25).

Many important questions arise:

1. Are the menstrual disturbances a consequence of the treatment?

Table II. *Complications in cases of secondary amenorrhea after stopping oral contraceptives*

During or after treatment with contraceptive pills	Amenorrhea spontaneous recovery >6<12 months N=38 (%)	>12 months N 67 (%)	Still amenorrheic >12 months N 72 (%)
Loss of weight	2.6	16.4	27.8
Gain of weight	7.9	20.9	16.7
Mental disturbances	—	7.4	15.1

struate after thyroid medication. 32 patients were very slim and had lost weight during and after treatment with contraceptive pills. Dieting is a very common cause of amenorrhea among girls in their early twenties. Among the patients who had lost weight 11 also had mental disturbances and had for a short time been treated at psychiatric hospitals. Another 5 patients of normal weight had been treated at psychiatric hospitals for mental disturbances. Two patients were slightly anaemic and 3 patients were menopausal. One patient suffered from diabetes. If we exclude these patient from the series, there are still 122 patients who have no other explanation for their amenorrhea than the use of contraceptive pills. 37 of them had earlier had children.

Twenty-nine patients increased in weight during and after treatment with contraceptive pills. Whether this increase of weight plays a role in the development of amenorrhea is not known. Many workers have noted relative impairment of glucose tolerance in women taking oral contraceptives. It has been suggested that this effect is due to an alteration of liver function, the production of ... cortisol or an alteration in the peripheral usage of glucose.

In all cases the radiological examination revealed a normal sella turcica and the fields of vision were normal.

2. What is the risk of developing amenorrhea following the use of contraceptive pills and which women will develop amenorrhea?

There is little information in the literature about the incidence of amenorrhea after the use of contraceptive pills. Whitelaw et al. (28) consider that there is a higher frequency of amenorrhea and anovulatory cycles after cessation of oral contraceptives than is generally believed. Rice-Wray et al. (22) have investigated 380 women who had stopped treatment with oral contraceptives in order to establish how soon ovulation returns to normal. They found that most women ovulated within 3 months, but 9 women (2.8%) had long periods of amenorrhea and in one case ovulation returned only after 390 days. Larsson-Cohn (18) studied a group of 515 patients and found that 4 of the subjects (0.8%) developed amenorrhea lasting more than 180 days. Bygdeman et al. (6) found that of 841 women who had stopped using contraceptive pills, 3 (2.7%) had amenorrhea for more than 6 months before menstruation returned. Among them 4 (0.6%) had amenorrhea for more than 12 months and one woman had persistent amenorrhea for more than 3 years at the time of the study.

In our group of 177 cases with amenorrhea after stopping contraceptive pills, altogether 63 cases (i.e. 35.4%) had had menstrual irregularities

Table III. *Irregular menstruation and pregnancies before treatment with contraceptive pills*

Before treatment with contraceptive pills	Amenorrhea spontaneous recovery 6–12 months N 38 (%)	12 months N 67 (%)	Persistent amenorrhea 12 months N 72 (%)
Irregular menses	44.7	29.9	36.1
Pregnancies	31.5	22.4	18.1

Table I. *Causes of secondary amenorrhea*

	Number	Spontaneously cured
Slight hypofunction of the hypothalamus	23	14 } 60%
Severe hypofunction of the hypothalamus	4	3
Chiari–Frommel syndrome	3	—
Amenorrhea after delivery	5	3
Premature menopause	14	—
Stein–Leventhal syndrome	1	—
Ovarian tumour	3	—
Anorexia nervosa	5	2
Amenorrhea after dieting	33	5
Obesity	6	1
Disturbances in the function of the uterus	5	4
Disturbances in the function of thyroid gland	5	—
Diabetes mellitus	2	—
Severe general disease	2	—
Total	143	

2. What is the risk of developing amenorrhea following the use of contraceptive pills and which women will develop amenorrhea?
3. How long will the amenorrhea last?
4. What is the precise cause of the amenorrhea?
5. What kind of treatment can be used?

A study was undertaken to clarify these problems as far as possible.

MATERIAL AND METHODS

49 patients, who during 1969–1971 were examined because of amenorrhea after the use of contraceptive pills, are studied. The patients came from different hospitals in Sweden, where they were examined by colleagues, or from Sabbatsberg Hospital in Stockholm, where they were examined by us. Hormonal excretion in the urine was determined at the Hormone Laboratory of the University Department of Obstetrics and Gynaecology, Sabbatsberg Hospital, Stockholm. All the patients had had amenorrhea for more than 6 months. A letter was sent to all patients in April 1972 with the following questions:

1. Has menstruation returned and, if so, when?
2. Is menstruation now regular?
3. Have you had any treatment since you left the hospital?
4. Have you given birth to a child since then, or are you pregnant now?

177 patients who answered the questions before May the first 1972 are included in this report.

The patients are divided into 3 groups. The first group includes 38 patients with amenorrhea lasting from 6–12 months and who had recovered spontaneously. In the second group 67 patients are included. They had had amenorrhea for 12–39 months and had recovered spontaneously. 72 patients who in May 1972 still had amenorrhea belong to group 3. All patients have been examined thoroughly at the different hospitals. In addition to general clinical and gynaecological examination the following laboratory tests were performed: Determination of electrolytes, haemoglobin, thrombocytes, white cell count, serum iron, mean corpuscular haemoglobin concentration (MCHC), cholesterol, serum proteins, lipids, plasma oxaloacetic transaminase (GOT), plasma pyruvic transaminase (GPT). The function of the thyroid gland with protein-bound iodine (PBI), triiodothyronine (T_3) and tetraiodothyronine (T_4) was investigated. Vaginal cytology was carried out. X-ray of the skull and sella turcica was performed. Determination of the visual fields was made. Histological examination of the endometrium was performed and in many cases the ovaries were examined by laparoscopy. Intravenous glucose tolerance test was performed on obese patients. The excretion in the urine of total gonadotropins, LH, low polar oestrogens, pregnanediol, 17-keto-steroids, fractionated 11-deoxy-17-ketosteroids and 17-ketogenic steroids was determined. The gonadotropins were estimated biologically on infantile mice by the method of Hamburger (15). The gonadotropin extracts from the urine were obtained as described by Johnsen (17). LH was determined by a radio-immunological method of Wide (29). For estimation of low polar oestrogens the method of Carlström & Furuhjelm (8) was used. 17-keto-steroids were determined according to Vestergaard (26), fractionated 11-deoxy 17-keto-steroids according to Carlström et al. (7). For the determination of 17-ketogenic steroids we used the method of Birke et al. (5).

RESULTS

1. *Are the menstrual disturbances a consequence of the treatment?*

Normal menstrual function depends upon the presence of a functional uterus and normal function of the ovaries, pituitary and hypothalamus. Amenorrhea and oligomenorrhea develop as a result of disturbances within this system. The disturbances may have different causes. There are no data available concerning the incidence of secondary amenorrhea in the normal population. The incidence of spontaneous amenorrhea in women at risk (i.e. exposed to certain stressful situations) has been estimated to be between 2 and 7% (Drew et al. (9)). In a series of 143 cases of secondary amenorrhea, not due to oral contraceptives, examined at our hospital between 1960 and 1967 (12) the cause for the amenorrhea was as listed in Table I.

In our present series of 177 patients with amenorrhea after they stopped taking contraceptive pills, two cases of hypofunction of the thyroid gland were found. The patients started to men

Table IV. *Hormonal excretion*

	Amenorrhea spontaneous recovery		Persistent amenorrhea
	> 6 < 12 months N 38 ( )	> 12 months N 67 ( )	> 12 months N = 72 (%)
Low polar oestrogens	31 6	50.7	72.8
17-ketosteroids	18.4	34.4	41 4
17-ketogenic steroids	26.3	25.3	34.3

of the patients who continued to have amenorrhea had an excretion of low polar oestrogens of less than 5 µg/24 hours. The repeated finding of low values of low polar oestrogen excretion in the urine is associated with a longer duration of the amenorrhea.

There is no difference between the three groups in the question of the age when they started to take contraceptive pills. In the groups of patients with spontaneous recovery 78 9 and 77 6 % respectively were less than 25 years of age and in the group of patients who still had amenorrhea, 80 % were less than 25 years.

4. *What is the precise cause of the amenorrhea.* The low excretion of gonadotropins and steroids in the urine together with other symptoms suggest that suppression of the hypothalamic releasing factors is the cause of the amenorrhea. It may be possible also that disturbances in the ovaries develop which prevent the ripening of follicles and the formation of corpora lutea. We have inspected the ovaries by laparoscopy or laparotomy in patients with amenorrhea lasting more than 1 months. In all cases we observed small inactive ovaries without any follicles or corpora lutea.

In 113 patients the excretion of 11-deoxy 17-ketosteroids in the urine was determined. No excretion of dehydroepiandrosterone (DHA) was detectable in 62 % of the cases. The theca cells of the ovaries produce most of the ovarian DHA. The lack of excretion of urinary DHA in cases with long-standing amenorrhea is therefore in good agreement with the absence of follicles and corpora lutea in the ovaries. In normally menstruating women, most of the urinary DHA seems to come from the ovaries and during menstrual cycles the urinary excretion of DHA follows that of pregnanediol (2). According to Maurais-Jarvis (20) lynestrol and other progestogens may influence the activity of the ovarian enzymes. A change in the activity of 17-β-hydroxysteroid dehydrogenase may develop and hence disturbances in the synthesis of steroid hormones appear. The transformation of pregnenolone to DHA is also inhibited. Appelgren (3) has recently shown that medroxyprogesterone and ethinyloestradiol inhibits the enzymatic activity which in the ovaries transforms pregnenolone to progesterone. According to Aak aag (1) chlormadinone inhibits the same enzyme system. There are consequently facts which support the theory that oral contra-

Table V. *The age of the patients when starting with contraceptive pills*

	Amenorrhea spontaneous recovery				Persistent amenorrhea	
	6 12 months		12 months		12 months	
Age	No.		No		No	%
15 19	16	42 1	27	40 3	22	30
20 24	1	36 8	25	37 3	36	50
25 29		13 2	14	20 9	12	17 1
30 34	2	5 3	—	—	1	1 4
35 39	1		1	1.5	1	1 4
Sum	8		47		72	

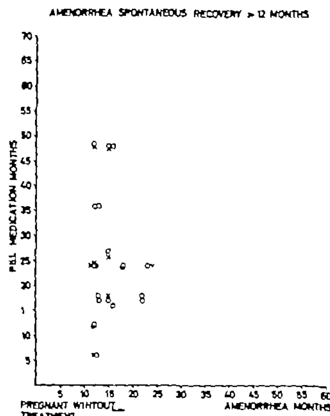


Fig 1 Correlation between length of treatment and length of amenorrhea in cases with spontaneous recovery

before using oral contraceptives. In Starup's (25) series of 31 patients with post "pill" amenorrhea, 55% had previous menstrual irregularities. Nillius (21) reports 43% and Larsson-Cohn (18) only *14.2% in his series.*

In a series of 2 262 primiparas investigated by Westman (27) the incidence of previous menstrual disturbances among them was only 7 7%. The incidence of irregular menstruation in nulliparous women is not known.

The women who get post "pill" amenorrhea consequently have a higher incidence of earlier menstrual disturbances than in ordinary cases. On the other hand in our series 20 8% had children and the rest had menstruated regularly. Thus it is impossible to foretell that a certain woman will develop this complication.

3 *How long will the amenorrhea last?*

In our series no correlation exists between the duration of oral contraceptive therapy and the length of the subsequent period of amenorrhea. 38 (21 4%) of the patients had amenorrhea for 6–12 months, and then menstruation returned spontaneously. In 67 patients (37 8%) the amenorrhea lasted for more than 12 months but they started to menstruate spontaneously or became pregnant and the mean duration of amenorrhea was 23 7 ± 1 8 months with the shortest duration of 12 months and the longest *of 39 months. The* mean period of treatment with pills in this group was 19.4 ± 0.9 months. Among the 7? patients (i.e. 40.8%) in whom amenorrhea persisted at the time of the study the mean period of "pill" taking was 20.2 ± 1.5 months, the mean duration of the amenorrhea was 29 6 ± 1 6 months and the longest spell of continuous amenorrhea was 5 years. In the thin patients the amenorrhea lasted for a mean period of 33 7 ± .5 months and in 65% of them menstruation had not returned. 11 women resumed contraceptive pills in spite of continuing amenorrhea.

In an earlier report of 30 patients with post "pill" amenorrhea we found a low excretion of steroids in the urine (11). Starup (25) also found a low excretion of oestrogen in his series of 31 patients. In the present study it is evident that a low excretion of low polar oestrogens and 17 ketogenic steroids are found in the groups with amenorrhea lasting more than 12 months. 72.8%

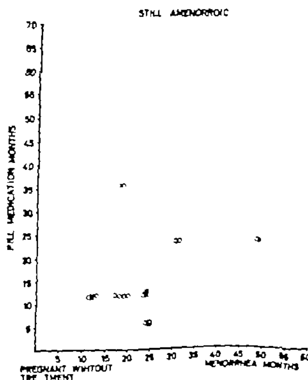


Fig Correlation between length of treatment and length f amenorrhea. Still amenorroic patients.

after treatment. Unfortunately her husband was found to be sterile.

Treatment with different kinds of steroid in higher doses should be avoided! As the patients already have suppression of the hypothalamic pituitary system it must be wrong to add to the existing inhibition, the inhibiting effect of further exogenous steroid. From our series it seems evident that treatment with steroids prolongs the amenorrhea rather than cures it.

REFERENCES

1. Adversy, A. Effekten av clomifendosen på konversion av pregnenolon till progesteron i homogenat av ovarier från ung. Nordisk fertilitetsklubbs 10: möte, Kristiansand, 1969.

Brown, H., Lindblom, T. & Svendborg, A. Simultaneous determination of urinary estrogens, pregnanediol 11-deoxy-17-ketosteroids, total 17-ketosteroids, and 17-ketogenic steroids, pregnanediol and plasma ... during the menstrual cycle and in early pregnancy. Am Med Exp Form 45, 777. 1967.

Sigtryggsson, L. E. Interference with progesterone effects of some antifertility compounds as shown ... biochemistry Congress Proceedings, Second European Congress on Sterility. Dubrovnik, 1969.

Stenson, D., Carlström, K. & Furuhjelm, M. Långvarig amenorré efter utlösandet av ovulationshämmare. Läkartidn 66 3365. 1969.

Lindqvist, G., Diczfalusy, E. & Plantin, L. O. Assessment of the functional capacity of the adrenal cortex. I. Establishment of normal values. J Clin Endocr 181, 98. 1958.

Södermark, M., Svensson, G. & Westerholm, B. Biverkningar hos ett p-pillerklientel med särskild hänsyn till blodkoagulationsrubbningar. Läkartidn 69 4. 1972.

Carlström, K., Furuhjelm, M., Ingelman-Sundberg, A. & Landgren, N. O. Gaschromatographic estimation of individual urinary 11-deoxy-17-ketosteroids. Int J Gynec Obstet 7 1-4, 1969.

Carlström, K. & Furuhjelm, M. A routine chemical method for the estimation of low polar oestrogens in human urine. Acta Obstet Gynec Scand 50 2 8, 1971.

Drew, F. L. & Stifel, E. N. Secondary amenorrhea among young women entering religious life. Obstet Gynec 32 4. 1968.

Friedman, S. & Goldfien, A. Amenorrhea and galactorrhea following the oral contraceptives. JAMA 19 1 48. 1969.

Furuhjelm, M., Carlström, K. & Ingelman-Sundberg, A. Longstanding amenorrhea following the use of contraceptive pills. Acta Europ Fertil 1 431. 1969.

Furuhjelm, M. Treatment of amenorrhea. Homenaje al profesor doctor Luis Canelas Ayala, XXV aniversario Profesional, M vica 11 75. 1970.

13. Gambrell, R. D. Jr., Greenblatt, R. B. & Mahesh, V. B. Post-pill-related amenorrhea-galactorrhea. Amer J Obstet Gynec 110 838, 1971.

14. Halbert, D. R. & Christian, C. D. Amenorrhea following oral contraceptives. Obstet Gynec 34 161. 1969.

15. Hamburger, C. Studies on gonadotrophic hormones from the hypophysis and chorionic tissue with special reference to their differences. Munksgaard, Copenhagen, 1933.

16. Homesley, H. D. & Goss, D. A. Menstrual dysfunction following use of oral contraceptives. Obstet Gynec 35 734. 1970.

17. Johnsen, S. G. A clinical method for the quantitative determination of gonadotrophins in 4-hrs samples of urine. Acta Endocr (Kbh) 28 69. 1958.

18. Larsson-Cohn, U. The length of the first three menstrual cycles after combined oral contraceptive treatment. Acta Obstet Gynec Scand 48 416, 1969.

19. MacLeod, S. C., Parker, A. S. & Perkin, J. A. The oversuppression syndrome. Amer J Obstet Gynec 106 359. 1970.

20. Mauvais-Jarvis, P. Metabolisme de la testostérone et du sulfate de déhydroepiandrostérone radioactifs, chez des sujets traités par le lynoestrénol, les oestrogènes de synthèse et leur association. Acta Endocr (Kbh) 57 37. 1968.

21. Nilhos, S. J. Amenorré efter behandling med perorala antikonceptionsmedel. Obstetrik och gynekologi 6 (ed C. Gemzell) 81. 1970.

22. Rice-Wray, E., Correu, S., Gorodovsky, J., Esquivel, A. & Goldzieher, J. W. Return of ovulation after discontinuance of oral contraceptives. Fertil Steril 18 212, 1967.

23. Shearman, R. P. Amenorrhea after treatment with oral contraceptives. Lancet 7473 1110. 1966.

24. Shearman, R. P. The investigation on treatment of amenorrhea developing after treatment with oral contraceptives. Int J Fertil 13 3 1. 1968.

25. Starup, J. Amenorrhea following oral contraception. Acta Obstet Gynec Scand 51 431. 1972.

26. Vestergaard, P. Rapid macro-modification of the Zimmerman Callow procedure for the determination of 17 ketosteroids in the urine. Acta Endocr (Kbh) 8 193. 1951.

27. Westman, A. The incidence of spontaneous cure of secondary amenorrhea and oligohypomenorrhea. Acta Obstet Gynec Scand 37 261. 1958.

28. Whitelaw, M. J., Nola, V. F. & Kalman, G. F. Irregular menses, amenorrhea and infertility following synthetic progestational agents. JAMA 1958 770, 1966.

29. Wide, L. & Porath, J. Radioimmunoassay of proteins with the use of sephadex-coupled antibodies. Biochem Biophys Acta 130 257. 1966.

Submitted for publication Dec. 21, 1972

M. plan. Furuhjelm
Department of Obstetrics and Gynecology
Sabbatsbergs sjukhus
Stockholm
Sweden

Table VI *Patients treated with steroids because of "pill" amenorrhea*

	Amenorrhea spontaneous recovery		Persistent amenorrhea
	>6-12 months N=38 (%)	>12 months N=67 (%)	>12 months N=72 (%)
Treatment with steroids	5.2	23.9	40.3

ceptives interfere with the function of the ovary itself as well as with the function of the hypothalamus.

It is more difficult for the organism to dispose of the synthetic hormones used in the oral contraceptives than the naturally occurring sex steroids. The possibility of accumulation of the synthetic steroids in the tissues must therefore be taken into consideration. Long term effect caused by accumulated synthetic steroids might be responsible for some cases of amenorrhea of shorter duration.

5 *Treatment*

It must be pointed out that there is a very strong tendency for a spontaneous remission of the secondary amenorrhea. In the series of Drew et al (9) 95% of the patients recovered without treatment within 12–18 months. Westman (27) concluded that in his series 80% recovered spontaneously. In our series of secondary amenorrhea (11) 47 women had a disturbance of hypothalamic pituitary function as the cause of their amenorrhea. 60% of them had a spontaneous remission.

In the literature some reports indicate that amenorrhea after stopping contraceptive pills responds to treatment with glucocorticoids. In none of our cases have we found anything to prove that a hyperfunction of the adrenals was present. Three of our patients had an elevated excretion of 17 ketosteroids with an elevated excretion of DHA. They were obese and after dieting, menstruation returned. Two of them became pregnant.

The indication for treating the patient is that she wants to have regular menstruations or if she wants to become pregnant. It is important to clarify the strength of the hypothalamic pituitary suppression.

1. If there is only suppression of the centre responsible for surges of gonadotropin release, the patients have a moderate secretion of oestrogens. The management in these cases should be expectant as the rate of spontaneous recovery is high. In our series 59.3% recovered spontaneously and among them 31 patients became pregnant without any treatment at the first ovulation after stopping the contraceptive pills.

If patients with a moderate secretion of oestrogens want to become pregnant they should be treated with clomiphene, either alone, or if necessary in combination with HCG. 14 of our patients wanted children and all of them responded to treatment with clomiphene alone and became pregnant.

2. If the centre responsible for a constant basal production of gonadotropins also is inhibited there is a low excretion of oestrogens and also often a low excretion of 17 ketosteroids. Patients who have an excretion of low polar oestrogens in the urine of less than 5 μg/24 hours on repeated examination or a vaginal smear without any oestrogenic influence should be treated with low doses of oestrogens (10–20 μg of ethinyl-oestradiol or 1–2 mg of oestradiol valeriate) for a long period of time. The oestrogen therapy should be given as substitution therapy in order to avoid atrophy of the genital organs. In many cases the uterus is very small probably as result of the anti-oestrogenic effects of the gestagens. Small doses of oestrogens also stimulate the excretion of the FSH-LH releasing hormones from the hypothalamus.

If patients with a low excretion of oestrogens want to become pregnant they should be treated with repeated injections of human menopausal gonadotropin (HMG) followed by one injection of 1?–18 000 I.U. chorionic gonadotropin (HCG). The ovaries respond to such a treatment given in sufficient doses by ovulation. One patient in our series wanted to become pregnant and ovulated

Acta Obstet Gynec Scand 52, 381–386, 1973

CONGENITAL ARRHYTHMIAS WITH SUPRAVENTRICULAR TACHYCARDIA IN THE PERINATAL PERIOD

Peter Herin and Claes Thorén

From Karolinska Institutet Department of Pediatrics (Head: Professor R. Zetterström), St Göran's Hospital for Children, Stockholm, Sweden

Abstract. Fourteen cases with perinatal tachyarrhythmias are described. Intra-uterine cardiac arrhythmia was observed in 8 cases, 6 with paroxysmal supraventricular tachycardia (PST) and 2 with atrial flutter, all but one with variable AV-block, and heart rate ranging between 2?0–3?0 beats/min. Caesarean section was performed because of suspected asphyxia in 4 cases and delivery by vacuum extraction in 2 of the 8 cases. All 6 cases first appearing in the first 4 days of life had PST with heart rate ranging from 210–310. Congestive heart failure, occurring in 10 cases in the total series, developed significantly earlier in the patients with intra-uterine diagnosed tachycardia. No cause was found in 10 cases, and only 2 cases displayed the Wolff-Parkinson-White (WPW) syndrome. The importance of early ECG recording in all newborns with arrhythmia is stressed. An increased use of the fetal ECG is recommended in cases with rapid or irregular fetal heart sounds. The delivery should be accelerated when the fetal heart rate is constantly more than 200 beats/min.

A fetal heart rate outside the normal range is often interpreted as a sign of asphyxia. This is also the case when the heart rhythm is irregular. Therefore it is not surprising that fetal arrhythmias due to heart disease, tachycardia and total AV-block, may lead the obstetrician to hasten delivery in the belief that the fetus is distressed. Many tachycardias are supraventricular and are often unstable because of varying AV-block (9). Various types of tachyarrhythmia in unborn children have been diagnosed by means of fetal electrocardiograms (3, 7, 13, 16) even in cases with such a rapid rate that no heart sounds could be auscultated (Bratteby 1972, personal communication).

Paroxysmal supraventricular tachycardia (PST) in early infancy is a well known and often dramatic condition, frequently leading to congestive heart failure (1). In a Swedish study of PST in infancy (11) 10 infants out of 54 were less than 1 week old and 6, by auscultation, had a rapid fetal heart rate in utero.

The purpose of this paper is to report on some diagnostic and therapeutic problems in 14 newborn infants with tachyarrhythmia.

MATERIAL AND METHODS

Over a period of 6 years, 13 newborn babies with congenital tachycardia were admitted to the paediatric clinic from other maternity hospitals; one was not diagnosed until the age of 6 months. Ten of the 14 infants were born in 1970–1972. They were divided into two groups having rapid or irregular heart rates, one group observed in utero and the other in the first post-partum days of life. The sex ratio was 6 males to 2 females in the prenatal group but 1 : 5 in the postnatal group. The gestational age in all cases was from 38–42 weeks and the median weight about 10% above average, although some of the infants displayed any apparent oedema. Individual data are given in Table I. Three children died (Nos. –, 6 and 8). The other 11 have been followed-up to the age of 3 years (average 14 months). The tachyarrhythmias were diagnosed according to the criteria given for infants by Lundberg.

RESULTS

Four of the babies with *intra-uterine tachycardia* were delivered by Caesarean section (CS) and by vacuum extraction (VE). The Apgar score in all cases but one (No.) was acceptable within 5 minutes after birth but definitely low one minute after birth in 4 cases (Nos. 2, 3, 4, 8). Within 1 to 6 hours after birth 5 children displayed symptoms of fulminating progressive heart failure: Pallor or cyanosis, tachypnoea, liver enlargement and cardiomegaly with pulmonary congestion on X-ray. ECG recordings made in 7 cases from 1 to

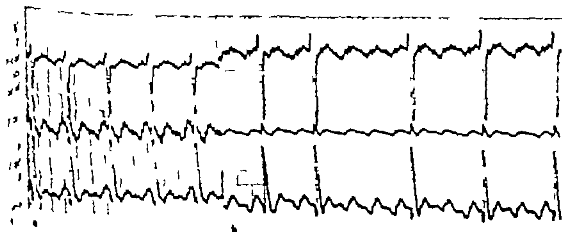
Follow up time, comment
Normal, 9? m
Dead 9 hr
Normal, 11 m
Normal, 20 m
Normal 6 m
Dead at [illegible]
[illegible], pulm. hypertension, right heart [illegible] fibroelastosis
Normal, 12 m
Dead [illegible]
[illegible]
Normal, 12 m
Normal, 6 m
Normal, 14 m, VSD (small ventricular septal defect)
Normal, 21 m
PST for 12 m
[illegible] rhythm, 19 m
Normal 3 m

ular septal defect of no haemodynamic significance no child in this postnatal group showed any signs of congenital heart malformations.

Digoxin (Lanoxin®) was administered intravenously to 8 of the 14 cases in an initial digitalizing dose of 0.08 mg/kg per day and Lanatoside (Cedilanid®) was administered i.v. to 4 cases in an equivalent dose of 0.02 mg/kg per day as the first drug of choice. In 3 cases (Nos. 2, 6 and 12) digitalis failed to inhibit the PST. Ajmalin, verapamil and propranolol, all administered i.v. also failed in case 12. Not even d.c. shock was able to convert the tachycardia of constant, ectopic supraventricular origin for 12 months but treatment kept the patient out of heart failure by producing an AV-block with a ventricular rate between 140–160 beats/min. In case 7 the tachycardia was converted into a sinus rhythm by d.c. cardioversion and in case 5 the tachycardia was spontaneously converted into a sinus rhythm; both cases were then digitalized.

DISCUSSION

Among the most important signs of imminent fetal asphyxia is a heart rate deviating from normal. However there is still no proper and simple method for distinguishing cardiac tachyarrhythmia from sinus tachycardia because of fetal distress. Thus 4 of the 8 prenatal cases in this series were



F[illegible] (a) Limb lead in case [illegible] h[illegible] of [illegible] birth showing supraventricular tachycardia [illegible] atrial flutter and [illegible] rate [illegible] beats [illegible]. (b) Two hours later: flutter rate unchanged at 404 beats/min but [illegible] varying degrees of AV-block, resulting in a change to ventricular bradycardia.

Table I Clinical data of 14 children with supraventricular tachycardia in the perinatal period

Patient no., sex	Fetal heart rate (beats/min)	Delivery	Apgar score (1 5 min)	Age at ECG-diagnosis	Type of tachycardia, heart rate (beats/min)	WPW	Heart failure	Therapy
Prenatal tachycardia								
1 M	160–200	VE	8–10	6 h	PST 2 1 block 160–200	–	+	Lanatocid C
2. M	140	NL	5–5	5 hr	PST 2 1 block 190 ventr fibrillation	?	+	Lanatocid C, no effect
3 M	180	VE	4–10	12 hr	PST 210	–	–	Digoxin
4 F	140–200	CS	4–10	1 hr	Flutter 2 3–4 1 block 120–220	–	+	Digoxin
5 F	100–180	CS	10–10	1 hr	PST variable block 170	–	–	Spontaneous conversion
6 M	200–250	CS	9–10	6 hr	PST variable block 150–230	–	+	Lanatocid C, Digoxin, no effect
7 M	140–175	NL	10–10	6 m	Flutter 2 3 1 block ventr fibrillation	–	–	DC
8. M	120–200	CS	4–9	4 hr	PST irregular 120–205	–	+	Digoxin
Postnatal tachycardia								
9 F	140	NL	10 10	4 d	PST variable block 310	+	+	Lanatocid C, Digitoxin
10 M	136	NL	10–10	6 d	PST 280	–	+	Digoxin
11 F	156–168	NL	10–10	4 d	PST 265	–	–	Digoxin
12. F	140	NL	10–10	3 d	PST variable block, 210	–	+	Digoxin, DC, Ajmalin Verapamil, no effect
13 F	144	NL	10–10	3 d	PST 260 ventr fibrillation	+	+	Digoxin
14 F	150	NL	10–10	3 d	PST 280	–	+	Digoxin

NL = Normal labor VE = Vacuum extraction, CS = Caesarean section, h = hour d day w = week, m = month, PST Paroxysmal supra-ventricular tachycardia DC = Direct current shock

12 hours after birth disclosed supraventricular tachycardia and varying degrees of AV-block resulting in an irregular ventricular rhythm Two of these cases (Nos. 4 and 7) were diagnosed as atrial flutter (Fig. 1) None of the 8 children displayed a WPW syndrom There were no congenital heart malformations among the cases with prenatal tachycardia

In the 3 cases which died the heart was grossly enlarged (Fig. 2) In case 2 an organic heart defect was suspected and during cardiac catheterization the baby died from ventricular fibrillation which could not be converted with defibrillation at the age of 9 hours. Patient no 6 died at 8 weeks of age with a persistent arrhythmia, despite treatment signs of pulmonary vascular obstruction and right atrial fibroelastosis were found post mortem. The third case (No. 8) reverted to sinus rhythm during digoxin therapy but with ECG signs of enormous ventricular hypertrophy The baby had convulsions and died at home. Post-mortem examination revealed a rhabdomyoma which earlier has been reported to be associated with tachyarrhythmia (17)

In the group diagnosed *postnatally* all the 6 children had a spontaneous vertex delivery after an uneventful labour and were asymptomatic for the first 3 days after birth. However 5 of them then developed heart failure. In all 6 cases the ECG showed supraventricular tachycardia with a ventricular rate ranging from 210–310 beats/min (mean 270) Two of them had a variable AV block but no atrial flutter or fibrillation In 2 cases pre-excitation (WPW syndrome) was recorded in ECG after conversion into sinus rhythm (Fig. 3). Apart from case 12 with a slight ventric

Follow up time, comment
Normal, 30 m
Died 9 hr
Normal, 31 m
Normal, 20 m
Normal 4 m
Died 1 [illegible] Autopsy, pulm. hypertension right heart failure atrial fibroelastosis
Normal 12 m
Died 4 m Rhabdomyoma
Normal, 32 m
Normal 6 m
Normal, 14 m, VSD (small ventricular septal defect)
Normal, 21 m
PST for 12 m
Several relapses, 19 m
Normal, 3 m

ular septal defect of no haemodynamic significance, no child in this postnatal group showed any signs of congenital heart malformations.

Digoxin (Lanoxin®) was administered intravenously to 8 of the 14 cases in an initial digitalizing dose of 0.08 mg/kg per day and Lanatoside (Cedilanid®) was administered i.v. to 4 cases in an equivalent dose of 0.02 mg/kg per day as the first drug of choice. In 3 cases (Nos. 2, 6 and 12) digitalis failed to inhibit the PST. Ajmalin, verapamil and propranolol, all administered i.v., also failed in case 12. Not even d.c. shock was able to convert the tachycardia of constant, ectopic supraventricular origin for 12 months but treatment kept the patient out of heart failure by producing an AV-block with a ventricular rate between 140–160 beats/min. In case 7 the tachycardia was converted into a sinus rhythm by d.c. cardioversion, and in case 5 the tachycardia was spontaneously converted into a sinus rhythm, both cases were then digitalized.

DISCUSSION

Among the most important signs of imminent fetal asphyxia is a heart rate deviating from normal. However there is still no proper and simple method for distinguishing cardiac tachyarrhythmia from sinus tachycardia because of fetal distress. Thus 4 of the 8 prenatal cases in this series were

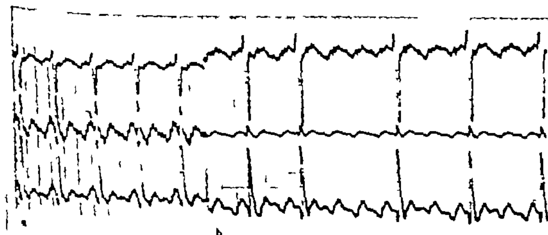


Fig. 1. (a) Limb lead in case 4, 1 hour after birth showing [illegible] tachycardia with atrial flutter and [illegible] ventricular rate of 202 beats/min. (b) Two hours later flutter are unchanged at 404 beats/min but with varying degrees of AV-block, resulting in change to ventricular bradycardia.



Fig 2 X-ray of case shows gross cardiomegaly 3 hours after birth. There was PST with a entricular rate of only 190 beats/min and a 2 1 block.

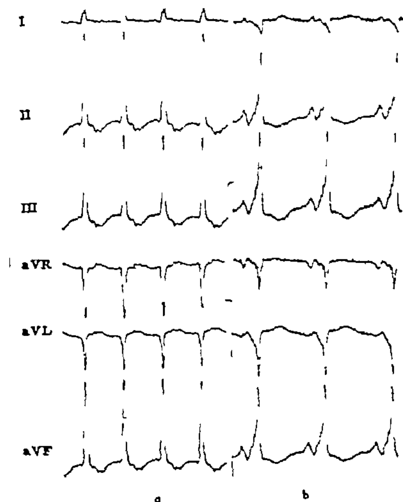


Fig 3 Limb lead in case 13, ( ) 3 days old during supraventricular tachycardia with rate f 760 beats/min. (*b*) Sinus rhythm 10 min after i.m. administration of 0.05 mg f digoxin, rat 155 be min, with pre-excitation = WPW syndrome characterized by shortened PR-i terval, ide and bizarre QRS complexes.

delivered by CS and two by VE because of imminent asphyxia. So the situation is still disturbing when a fetus has a high or irregular heart rate. In a study of 112 Stockholm cases of imminent fetal asphyxia delivered by Caesarean section, Fursbyhn (4) also found that 36% of the infants had a rapid or irregular heart rate.

In those cases where an abnormal heart rate is due to a cardiac arrhythmia, e.g. PST or atrial flutter the situation may be somewhat different, as some infants seem to tolerate the rapid heart rate in utero for rather a long time, even those (Nos. 1, 4, 6, 8) who developed postnatal heart failure. The reason for the greater tolerance in utero is not completely clear but in this situation the fetal circulation may be less of a loading factor than after birth when the two ventricles are pumping in series, thereby imposing greater demands on the left and weaker ventricle.

The symptomatic period after birth was brief in our 5 prenatal cases 6 hours or less. However after this interval, which was significantly shorter than in the postnatal group, the risk of overt symptoms of heart failure appears to be rather great. These symptoms all developed quite suddenly and the babies were very soon in poor condition with tachypnoea and cyanosis. Heart failure was more common (10 14) in this study than might be expected if the PST had appeared later in infancy or childhood (1, 11, 1). Of course many factors influence the development of heart failure factors such as duration and heart rate.

Several cases have been reported with proven tachycardia lasting several weeks up to 6 months prior to labour and in which heart failure did not develop until a few hours after birth (6, 9, 14). But there have also been neonates with extensive oedema and gross heart enlargement as evidence of intra-uterine heart failure (5, 8, 15) in cases and 8 in our series. Thus fetal tachycardia may very well be suspected of inducing fetal heart failure with a risk of intra-uterine death. By personal communication Bratteby reported one child dying with intra-uterine tachycardia established by ECG with heart rate of 350-390 beats/min in the 36th gestational week.

A PST with AV-block may remain undetected and the slower ventricular rate gives much less ... tachycardia. As shown in Fig. 1 ... atrial flutter with constant AV-block can never be diagnosed without an ECG. This explains why case 7 was not detected earlier than 6 months after birth, despite indications of prenatal tachycardia. A similar case has been reported (2).

In some cases the tachycardia may terminate spontaneously (10, 12), as in case 5 in this study. The vagal stimuli evoked by pharyngeal suction through a catheter may inhibit as PST in a newborn, which has been noticed (5).

CONCLUSIONS

What reasonable cardiological investigations can be performed to help the obstetrician determine whether induced or normal labour is indicated? Continuous monitoring of the fetal heart rate can indicate possible variations and their relation to the uterine contractions, the mother's position, her smoking habits etc. Tachycardia due to cardiac disease should be suspected if a continuous rapid rate of more than 200 beats/min is noted. Fetal ECG recordings especially from scalp electrodes could be of great diagnostic help particularly in those cases with irregular tachycardia, which may be misinterpreted as signs of fetal asphyxia. This study also shows that, in perinatal tachyarrhythmia, AV-block is common and there is a high risk of heart failure. Until a sufficiently refined and simple method comes into use for the determination of fetal asphyxia, active obstetrical intervention is still indicated. Early diagnosis of arrhythmias using ECG soon after delivery and early therapy for the arrhythmia to prevent the rapid development of heart failure are equally important.

REFERENCES

1. Anderson, R. C. & Adams, F. H.: Congenital paroxysmal tachycardia. J Pediat 43: 668, 1953.
2. Aaroltanen, J. & Samila, S.: Congenital atrial flutter. Report of a case. Acta Paediat Scand 59: 587, 1970.
3. Fluenenthal, S., Jacobs, J. C., Steer, C. M. & Williamson, S. W.: Congenital atrial flutter. Report of a case documented by intrauterine electrocardiogram. Pediatrics 41: 659, 1968.
4. Fursbyhn, M.: Caesarean section in cases of imminent fetal asphyxia. Acta Obstet Gynec Scand 49: 299, 1970.
5. Hedvall, G.: Three cases of paroxysmal tachycardia with onset in utero. To be published in Acta Paediat Scand, 1973.



Fig 2 X-ray of case 2 shows gross cardiomegaly 3 hours after birth. There was PST with a ventricular rate of only 190 beats/min and a 2 1 block.

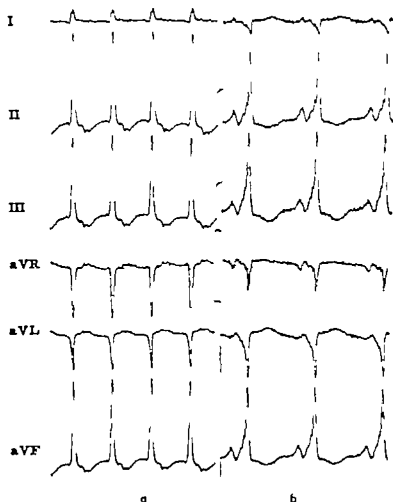


Fig 3 Limb lead in case 13 (*a*) 3 d old during supraventricular tachycar with a rate f 260 beats/min. (*b*) Si rhythm 30 min after i.m. administration 0 05 mg f digoxin, rate 155 bea /min, w pre-excitation – WPW syndrome charac rized by shortened PR-interval, wide a bizarre QRS complexes.

delivered by CS and two by VE because of imminent asphyxia. So the situation is still disturbing when a fetus has a high or irregular heart rate. In a study of 112 Stockholm cases of imminent fetal asphyxia delivered by Caesarean section Furuhjelm (4) also found that 36% of the infants had a rapid or irregular heart rate.

In those cases where an abnormal heart rate is due to a cardiac arrhythmia, e.g. PST or atrial flutter the situation may be somewhat different, as some infants seem to tolerate the rapid heart rate in utero for rather a long time even those (Nos 1 4 6, 8) who developed postnatal heart failure. The reason for the greater tolerance in utero is not completely clear but in this situation the fetal circulation may be less of a loading factor than after birth when the two ventricles are pumping in series, thereby imposing greater demands on the left and weaker ventricle.

The asymptomatic period after birth was brief in our 5 prenatal cases 6 hours or less. However after this interval, which was significantly shorter than in the postnatal group the risk of overt symptoms of heart failure appears to be rather great. These symptoms all developed quite suddenly and the babies were very soon in poor condition with tachypnoea and cyanosis. Heart failure was more common (10 14) in this study than might be expected if the PST had appeared later in infancy or childhood (1 11 12). Of course many factors influence the development of heart failure factors such as duration and heart rate.

Several cases have been reported with proven tachycardia lasting several weeks up to 6 months prior to labour and in which heart failure did not develop until a few hours after birth (6 9 14). But there have also been neonates with extensive oedema and gross heart enlargement as evidence of intrauterine heart failure (5 8 15) as in cases ... in our series. Thus fetal tachycardia may ... well be suspected of inducing fetal heart failure with risk of intra-uterine death. By personal communication Bratteby reported one ... dying with intra-uterine tachycardia, established by ECG with heart rate of 340–380 beats/min in the 36th gestational week.

A PST with AV-block may remain undetected and the slower ventricular rate gives much less concern than continuous tachycardia. As shown in the atrial flutter with constant AV-block can never be diagnosed without an ECG. This explains why case 7 was not detected earlier than 6 months after birth, despite indications of prenatal tachycardia. A similar case has been reported (2).

In some cases the tachycardia may terminate spontaneously (10 12), as in case 5 in this study. The vagal stimuli evoked by pharyngeal suction through a catheter may inhibit as PST in a newborn, which has been noticed (5).

CONCLUSIONS

What reasonable cardiological investigations can be performed to help the obstetrician determine whether induced or normal labour is indicated? Continuous monitoring of the fetal heart rate can indicate possible variations and their relation to the uterine contractions, the mother's position, her smoking habits etc. Tachycardia due to cardiac disease should be suspected if a continuous rapid rate of more than 200 beats/min is noted. Fetal ECG recordings especially from scalp electrodes could be of great diagnostic help particularly in those cases with irregular tachycardia, which may be misinterpreted as signs of fetal asphyxia. This study also shows that, in perinatal tachyarrhythmia, AV-block is common and there is a high risk of heart failure. Until a sufficiently refined and simple method comes into use for the determination of fetal asphyxia, active obstetrical intervention is still indicated. Early diagnosis of arrhythmias using ECG soon after delivery and early therapy for the arrhythmia to prevent the rapid development of heart failure are vitally important.

REFERENCES

1. Anderson, R. C. & Adams F. H. Congenital paroxysmal tachycardia. J Pediat 43 668 1953
2. Anttolainen J. & Simila, S. Congenital atrial flutter report of a case. Acta Paediat Scand 59 587 1970
3. Blumenthal, S. Jacobs, J. C. Steer C. M. & Williamson, S. W. Congenital atrial flutter report of a case documented by intrauterine electrocardiogram. Pediatrics 41 659 1968
4. [illegible], M. Caesarean section in cases of imminent foetal asphyxia. Acta Obstet Gynec Scand 49 ... 1970
5. Hedvall, G. Three cases of paroxysmal tachycardia with onset in utero. To be published in Acta Paediat Scand 1973

6. Hilrich, N. M. & Evrad, J. R. Supraventricular tachycardia in the newborn with onset in utero. Amer J Obstet Gynec *70* 1139 195?
7. Hon, E. H. & Quilligan, E. J. Electronic evaluation of fetal heart rate. Clin Obstet Gynec *11* 14 1968.
8. Kesson, C. W. Foetal paroxysmal auricular tachycardia. Brit Heart J 20 552, 1958
9. Leskoff, A. H. Perinatal outcome of paroxysmal tachycardia of the newborn with onset in utero. Amer J Obstet Gynec *104* 73 1969
10. Linde, L. M., Turner S. W. & Awa, S. Present status and treatment of paroxysmal supraventricular tachycardia. Pediatrics *50* 127 1972.
11. Lundberg, A. Paroxysmal tachycardia in infancy. A clinical and experimental study. Acta Paediat Scand, Suppl. 143 1963
12. Nadas, A. S., Daeschner C. W. Roth, A. & Blumenthal, S. L. Paroxysmal tachycardia in infants and children. Pediatrics *9* 167 195.
13. Shenker L. Foetal electrocardiography. Obstet Gynec Survey *21* 367 1966
14. Silendes, L. E., Anderson, N. A., Rachlin, A. & ... wark, N. J. Unusual auricular flutter in newborn infants. Report of a case and review of the literature. J Pediat *1* 43 195?
15. Silber, D. L. & Durnin, R. E. Intrauterine atrial tachycardia associated with massive oedema in newborn. Amer J Dis Child *11* 722, 1969
16. Straede Nielsen, J. & M...strup, J. K. Foetal electrocardiographic studies of cardiac arrhythmias and heart rate. Acta Obstet Gynec Scand 47 47 1968
17. Wegman, M. E. & Egbert, D. S. Congenital rhabdomyoma of the heart associated with arrhythmia. Pediat *6* 818 1935

Submitted for publication Jan. 5 1973

Claes Thorén
Division of Cardiology
S:t Görans barnsjukhus
S-11 81 Stockholm
Sweden

Acta Obstet Gynec Scand 52, 387–389 1973

CASE REPORTS

HIDROADENOMA OF THE VULVA

Niels Chr Nielsen

From the Department of Obstetrics and Gynaecology (Heads: G. Stakemann, M.D. and F. Lundvall, M.D.), Øresundshospitalet, Copenhagen, Denmark

[illegible] The microscopic appearances of [illegible] hidroadenoma are described. Differential diagnostic problems are discussed, emphasizing in particular the possibility of confusing the tumour with epidermoid carcinoma. One such case is reported. It is stressed that hidroadenoma is a benign tumour to be treated by wide local excision, unlike the malignant tumours which require total vulvectomy and excision of the lymph nodes.



Fig. 1. Hidroadenoma vulvae.

Hidroadenoma of the vulva was previously considered an extremely rare tumour. From the time of its first identification and description in 1904 (7) up to 1939 (8) only about 40 cases were reported. To-day about 200 cases are on record, but presumably a much larger number has occurred.

The reason why attention is now being called to this tumour again in connection with a single case report is the differential diagnostic difficulties that it may cause. Grossly hidroadenoma of the vulva may be mistaken for polypous carcinoma, and microscopically it may be confused with epidermoid carcinoma.

CASE REPORT

A [illegible]-year-old woman was admitted as a case of vulvar carcinoma. On the basis of a biopsy removed by the [illegible] own doctor the following diagnosis had been [illegible]: [illegible] carcinoma of the vulva derived from stratified squamous epithelium of the [illegible]-cell type. Gynaecological examination showed normal conditions apart from a [illegible] tumour 2 × 1 × 1 cm on the edge of the labium [illegible]. The tumour was covered with skin, freely [illegible] (Fig. [illegible]). There was no deep infiltration. No palpable [illegible] nodes. Although radical operation with [illegible] nodes might have appeared indicated on the basis of the microscopic report, the gross appearances [illegible] the microscopic report [illegible]. This showed clear-cell hidroadenoma [illegible].



Fig. 2. Hidroadenoma vulvae, removed by local excision.

6. Hilrich, N M & Evrad, J R. Supraventricular tachycardia in the newborn with onset in utero. Amer J Obstet Gynec *70* 1139 1955
7 Hon, E. H. & Quilligan, E. J Electronic evaluation of fetal heart rate Clin Obstet Gynec *11* 145 1968
8 Kerson, C. W. Foetal paroxysmal auricular tachycardia. Brit Heart J *20* 552, 1958
9 Levkoff A. H. Perinatal outcome of paroxysmal tachycardia of the newborn with onset in utero. Amer J Obstet Gynec *104* 73 1969
10. Linde L. M Turner S. W & Awa, S. Present status and treatment of paroxysmal supraventricular tachycardia. Pediatrics *50* 1 7 1972.
11 Lundberg, A. Paroxysmal tachycardia in infancy A clinical and experimental study Acta Paediat Scand, Suppl. 143 1963
12. Nadas, A. S., Daeschner C. W Roth, A. & Blumenthal, S. L. Paroxysmal tachycardia in infants and children. Pediatrics 9 167 195_
13 Shenker L. Foetal electrocardiography Obstet Gynec Survey *21* 367 1966.
14 Siderides, L. E., Antonius, N A., Richlan, A. & wark, N J Unusual auricular flutter in new infants. Report of a case and review of the litera J Pediat *31* 435 1937
15 Silber D L & Durnin, R. E. Intrauterine tachycardia associated with massive oedema newborn. Amer J Dis Child *117* 722, 1969.
16. Straede Nielsen, J & Moestrup, J K. Foetal elec cardiographic studies of cardiac arrhythmias and heart rate Acta Obstet Gynec Scand *47* 247 196
17 Wegman, M. E. & Egbert, D S. Congenital rh domyoma of the heart associated with arrhythmia Pediat 6 818 1935

Submitted for publication Jan 25 1973

Claes Thorén
Division of Cardiology
S t Görans barnsjukhus
S-11 81 Stockholm
Sweden

Acta Obstet Gynec Scand 52. 337–339 1973

CASE REPORTS

HIDROADENOMA OF THE VULVA

Niels Chr Nielsen

From the Department of Obstetrics and Gynaecology (Heads: G. Stakemann, M.D. and F. Lundvall, M.D.), Øresundshospitalet, Copenhagen, Denmark

Abstract. The microscopic appearances of vulval hidradenoma are described. Differential diagnostic problems are discussed, emphasizing in particular the possibility of mistaking this tumour with epidermoid carcinoma. One such case is reported. It is stressed that hidroadenoma is a benign tumour to be treated by wide local excision, and that it is not as malignant tumours which require total vulvectomy with dissection of the lymph nodes.

Hidradenoma of the vulva was previously considered an extremely rare tumour. From the time of its first identification and description in 1904 (7) up to 1939 (8) only about 40 cases were reported. To-day about 200 cases are on record, but presumably a much larger number has occurred.

The reason why attention is now being called to this tumour again in connection with a single case report is the differential diagnostic difficulties that it may cause. Grossly hidroadenoma of the vulva may be mistaken for polypous carcinoma, and microscopically it may be confused with epidermoid carcinoma.



Fig. 1. Hidroadenoma vulvae.

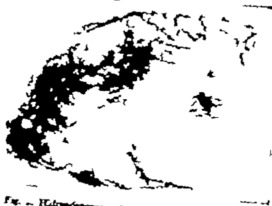


Fig. 2. Hidroadenoma vulvae, removed by local excision.

CASE REPORT

A 7? year-old woman was admitted as a case of vulval carcinoma. On the basis of a biopsy removed by the patient's own doctor, the following diagnosis had been made: Solid carcinoma of the vulva derived from stratified squamous epithelium of the clear-cell type. Gynaecological examination showed normal conditions apart from a tumour 2 × 1.5 × 1 cm, on the edge of the labium minus (Fig. 1). The tumour was covered with skin, freely movable, and there was no deep infiltration. No palpable inguinal nodes. Although radical operation with node dissection might have appeared indicated on the background of the microscopic report, the gross appearance made it reasonable first to await the microscopic report on frozen section. This showed clear-cell hidroadenoma

6. Hilrich, N M & Evrad, J R. Supraventricular tachycardia in the newborn with onset in utero. Amer J Obstet Gynec 70 1139 1955
7 Hon, E. H. & Quilligan, E. J Electronic evaluation of fetal heart rate Clin Obstet Gynec 11 145 1968
8. Kenson, C. W Foetal paroxysmal auricular tachycardia. Brit Heart J 20 552, 1958
9 Levkoff A. H Perinatal outcome of paroxysmal tachycardia of the newborn with onset in utero. Amer J Obstet Gynec 104 73 1969
10. Linde L. M Turner S. W & Awa, S.. Present status and treatment of paroxysmal supraventricular tachycardia. Pediatrics 50 127 1972.
11 Lundberg, A. Paroxysmal tachycardia in infancy A clinical and experimental study Acta Paediat Scand, Suppl. 143 1963
12. Nadas, A. S., Daeschner C. W Roth A. & Blumenthal, S. L. Paroxysmal tachycardia in infants and children. Pediatrics 9 167 195_
13 Shenker L. Foetal electrocardiography Obstet Gynec Survey 1 367 1966.
14 Siderides, L. E., Antonius, N A., Richlan, A. & wark, N J Unusual auricular flutter in newb infants. Report of a case and review of the literat J Pediat 51 435 1957
15 Silber D. L. & Durnin, R. E Intrauterine at tachycardia associated with massive oedema in newborn. Amer J Dis Child 117 722, 1969.
16. Straede Nielsen, J & Moestrup, J K. Foetal elec cardiographic studies of cardiac arrhythmias and heart rate. Acta Obstet Gynec Scand 47 47 1968
17 Wegman, M E. & Egbert, D S. Congenital rh domyoma of the heart associated with arrhythmia Pediat 6 818 1935.

Submitted for publication Jan. 25 1973

Claes Thorén
Division of Cardiology
S t Görans barnsjukhus
S-112 81 Stockholm
Sweden

Acta Obstet Gynec Scand 52: 387–389 1973

CASE REPORTS

HIDROADENOMA OF THE VULVA

Niels Chr Nielsen

From the Department of Obstetrics and Gynaecology (Heads G. Stakemann M.D. and F. Lundwall, M.D.), Øresundshospitalet, Copenhagen, Denmark

[illegible] The microscopic appearances of vulval hidro[illegible] are described. Differential diagnostic problems are [illegible], emphasizing in particular the possibility of [illegible] the tumour [illegible] epidermoid carcinoma. One such case is reported. It is stressed that hidroadenoma is a benign tumour to be treated by wide local excision, unlike the malignant tumours which require total vulvectomy [illegible] of the lymph nodes.

[illegible] of the vulva was previously considered an extremely rare tumour. From the time of its first identification and description in 1904 (I) up to 1939 (II) only about 40 cases were reported. To-day about 200 cases are on record, but presumably a much larger number have occurred.

The reason why attention is now being called to this tumour again in connection with a single case report is the differential diagnostic difficulties that it may cause. Grossly hidroadenoma of the vulva may be mistaken for polypous carcinoma, and microscopically it may be confused with epidermoid carcinoma.



Fig 1 Hidroadenoma vulvae



Fig 2 Hidroadenoma vulvae, removed by local excision.

CASE REPORT

A 77-year-old woman was admitted as a case of vulval carcinoma. On the basis of a biopsy removed by the patient's own doctor, the following diagnosis had been made: [illegible] carcinoma of the vulva derived from stratified squamous epithelium of the clear-cell type. Gynaecological examination showed normal conditions apart from a tumour 2 × 1.5 × 1 cm, on the edge of the labium [illegible] (Fig. 1). The tumour was covered with skin, freely movable, and there was no deep infiltration. No palpable [illegible] nodes. Although radical operation with node [illegible] might have appeared indicated on the basis of the microscopic report, the gross appearances [illegible] it seemed advisable first to await the microscopic report [illegible]. This showed clear-cell hidroadenoma

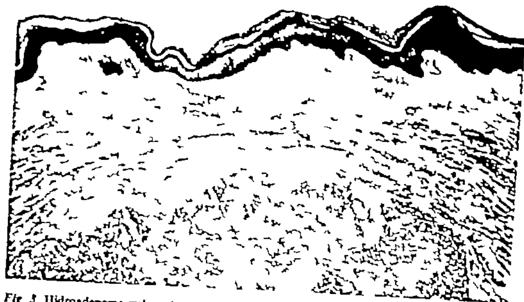


Fig 3 Hidroadenoma vulvae (×40).



Fig 4 Hidroadenoma vulvae (88).

of the vulva, a diagnosis which was subsequentl firmed by examination of the specimen removed b local excision (Fig.) It showed on microscopy defined tumour covered with a slightly acanthotic fied squamous epithelium (Figs. 3 and 4). The tumour composed of epithelial cells and was partially lobe The cells were uniform, polygonal, with distinct limits, a very pale cytoplasm, nd fairly unif rm, nuclei (Fig. 5).

The postoperative course was uneventful. More th months late the patient died f a cerebral injury Au showed no signs of recurrence

DISCUSSION

The gross appearances in the reported case w typical, but as already mentioned there may changes giving rise to an erroneous diagnosis. a case described by Hertig & Mansell (1) t polypous tumour had penetrated the overlyi epithelium and thus looked like a polypous ca cinoma.

The microscopic appearances may vary Her the findings will be mentioned only briefly b the interested reader is referred to Novak & Ste venson (5) and Novak & Woodruff (6) As a rul the hidroadenomas are well-defined, situated i the dermis, surrounded by a pseudocapsule of con nective tissue. In most cases there is a communi cation with the excretory duct of an apocrine sweat gland. The tumour is often of an adenoma tous appearance showing more or less dilated tu-



Fig. 3. Clear-cell hidroadenoma vulvae (× 250).

bles lined with simple or stratified columnar epithelium. A number are papillomatous, others solid adenomas consisting of columnar to cuboidal cells (4). Some hidroadenomas of the vulva, like the present case, show typical, large, pale cosmophilic cells of the appearance described for clear-cell hidroadenomas of the skin in general. The nuclei are large, sometimes irregular, and pearl-like formations may be present. Thus, in some cases the appearances may be highly suggestive of squamous-cell carcinoma. Indeed, McDonald et al. (4) described a large number of vulvar tumours which they designated "apocrine sweat gland carcinomas" but on subsequent revision these diagnoses are extremely questionable (2, 5). The diagnosis is very important, as clear-cell hidroadenoma of the vulva must be considered a *definitely* benign disease. A differential diagnostic possibility one must bear in mind the fairly uncommon Paget disease of aberrant mammary tissue situated along the nipple line, but non-pigmented malignant melanoma and sebaceous carcinoma may also give rise to confusion (3).

CONCLUSION

In diagnosing vulvar lesions the rare hidroadenoma must be borne in mind. This is a benign tumour although the varying microscopic appearances may give rise to confusion with carcinoma. The treatment of the benign vulvar hidroadenoma is local excision of the tumour, whereas the malignant varieties of vulvar tumours require radical operation with node dissection.

ACKNOWLEDGEMENT

Thanks are due to Professor H. Poulsen, Department of Pathology, Copenhagen City Hospital, for making the photomicrographs.

REFERENCES

1. Hertz, A. T. & Murrell, H. Pathology (ed. W. A. D. Anderson), Mosby, St. Louis, 1957.
2. Lennox, B. J Path Bact 67 553, 1954.
3. Lundvall, F. Cancer of the vulva (Thesis). Copenhagen, 1961.
4. McDonald, J. R., Lovelady, S. B. & Waugh, J. M. Amer J Obstet Gynec 42 306, 1941.
5. Novak, E. R. & Stevenson, R. R. Amer J Obstet Gynec 50 641, 1945.
6. Novak, E. R. & Woodruff, J. D. Gynecologic and Obstetric Pathology. Saunders, Philadelphia and London, 1967.
7. Pick, L. Virchows Arch Path Anat 175 312, 1904.
8. Rothman, D. & Gray, S. H. Amer J Obstet Gynec 38 509, 1939.

Submitted for publication Nov. 8, 1972

Niels Chr. Nielsen
Ole Nielsen 43 A
2800 Lyngby
Denmark

Acta Obstet Gynec Scand 52: 391–393 1973

A QUIET TYPE I HYPERLIPIDEMIA IN PREGNANCY

Acta Obstet Gynec Scand 52 391–393 1973

A QUIET TYPE I HYPERLIPIDEMIA IN PREGNANCY

Review and Report of a Case

L. R. Martinez, V A. Degennaro and E. J Veloso

From the Respiratory Intensive Care Unit of the State University Kings County Hospital Center (Head Leonardo R. Martinez M.D.) Brooklyn, New York, USA

Abstract Hyperlipoproteinemia is a condition characterized by increased lipid content of the blood. It may occur as a primary familial disease or as secondary to various clinical conditions such as seen in systemic lupus, alcoholism and liver disease. Of the primary forms of hyperlipoproteinemias, Type 1 is the least common and to our knowledge this condition has not been previously reported in pregnancy. The present report deals with Type I Hyperlipoproteinemia in 32 weeks pregnant patient, confirmed by electrophoresis study.

Hyperlipoproteinemia as a clinical phenomenon has attracted increasing attention in recent years. The primary Type I pattern as defined by Fredrickson and his associates (1) is extremely rare but recently the Type I pattern has been noted to occur in hypothyroidism, acute alcoholism and systemic lupus erythematosus. To our knowledge however there have been no cases reported in pregnancy. The patient to be discussed presented during pregnancy several features of familial Type I hyperlipoproteinemia, including the characteristic Type I electrophoretic pattern and resistance to heparin. Since the disorder is rare, a brief review of the literature and possible pathophysiology demonstrated in this patient is presented.

CASE HISTORY

The patient was 33-year-old West Indian female admitted to Kings County Hospital in Brooklyn, New York on 30 11 71 in an unconscious state with history of being found behind locked bathroom door. The patient, on admission, had an intra-uterine pregnancy of 32 weeks. The prenatal course had been previously uneventful and on 3 pre-natal visits she had been found normotensive and without glycosuria or proteinuria.

Her past history included an uneventful pregnancy in 1971 and no other known illnesses. Family history was unobtainable.

On admission B.P. was 140/90, Resp. Rate 24, pulse 110/regular, Temp. 100.4 F. The patient was comatose, pupils 3–4 cm and briskly reactive. Spastic rigidity with DTR 3+ were noted as well as bilateral Babinski signs. Heart and lungs were normal, extremities normal. The patient was convulsing actively for which $MgSO_4$ (10G) was given with no response, followed by Valium (10 mg) and Dilantin (250 mg) IV which caused cessation of convulsions. Admission laboratory values showed Hct. 28, Na 138, K. 4.5, Cl 103, CO_2 16, BUN 30, Glucose 160; urinalysis showed no glucose or acetone but 3+ protein.

Funduscopic examination revealed no papilledema but white globules and streaks were seen running through the retinal vessels. Spinal tap was performed; opening pressure 135 mmH_2O. The CSF fluid was clear, contained no cells, protein 23, glucose 130.

The patient developed Cheyne-Stokes respiration and was intubated via nasotracheal tube but allowed to breathe spontaneously. Blood gases showed pH 7.37 pCO_2 43 pO_2 74.

Ten hours after admission the patient spontaneously delivered an 1875 g live baby boy with an Apgar of 1. Because of poor respirations the patient was placed on Bird respirator and her ventilation was assisted.

The patient's blood was noticed to be opalescent and the plasma on standing contained a creamy supernatant layer. Lipid studies were done, showing Type I hyperlipoproteinemia electrophoretic pattern with broad band at the origin, elevated cholesterol and markedly elevated triglyceride. Lupus prep was negative, serum amylase 25 salicylate < 5 mg% and amobarbital 1 mg% (received in hospital). The patient continued to have cloudy plasma throughout her course. Pertinent lab results tabulated are shown in Table I.

On the second hospital day she became flaccid and areflexic with pupils sluggishly reactive to light. Respiration and blood pressure were maintained artificially. On the 3rd hospital day pupils became dilated and non-reactive and the patient became hypothermic (94°F).

Table I *Pertinent laboratory data*

	10/11/71	10/12/71	10/13/71	10/14/71
Na	138	128	147	142
Glucose	160	135	80	130
BUN	*30*	*15*	*18*	25
Creatinine	1.8	1 8	3.9	
Osmolarity serum		273	282	299
Urine		451	146	150
				post. *lipoprotein* lipase
Cholesterol	190	628	420	225
Triglyceride	950	500	330	145
PHLA	0 056 mEq/ mlFFA		(N = 0.24–0.40 mEq/ml FFA)	
FFA (mEq/l)			530	500

Electrophoresis showed Type I pattern, with broad band at origin

Blood pressure could no longer be maintained and EEG reading was flat. The patient remained essentially unchanged and suffered cardiac arrest on the fifth hospital day

DISCUSSION

The clinical problem of hyperlipoproteinemia is a complicated one involving the interplay of several mechanisms. Hyperlipoproteinemia may be primary or secondary The five primary types and the mechanisms behind their production have been described by Fredrickson and his co-workers (1 2 3) Secondary forms of hyperlipoproteinemia have been seen in a variety of clinical conditions including *myxedema obstructive liver disease oral* contraceptive use systemic lupus, hepatoma nephrotic syndrome myeloma, lymphoma and excessive alcohol ingestion.

Of the primary *forms Type I appears to be the* least common. This disorder is familial and in the homozygous state usually has its onset early in life Clinically the patients suffer from xanthomas of the eruptive type, lipemia retinalis, hepatosplenomegaly pancreatitis and recurrent bouts of abdominal pain The blood is opalescent and the plasma when left standing shows a creamy white supernatant layer *The lipid pattern is* characterized by markedly elevated triglycerides, mildly elevated cholesterol and slightly decreased fatty acids. On electrophoresis chylomicrons predominate (in the fasting state) and pre-beta lipoproteins may also be elevated. α and β lipoproteins are usually reduced.

The disorder also called fat induced hyperlipemia" is believed to result from a deficiency of lipoprotein lipase the enzyme which catalyses the breakdown of the triglycerides in chylomicrons (4) The diagnosis depends in addition to chemical *findings, on three factors: first is the finding of* the Type I pattern on protein electrophoresis, second is the demonstration of the relationship of dietary fat to the increase chylomicronemia *and third is the demonstration of low levels of* post heparin lipolytic activity (PHLA). This test, done in vitro on blood is based on the fact that lipoprotein lipase is activated by heparin.

In considering the patient presented here one must interpret the data in view of the fact that she was pregnant and that she apparently suffered from eclampsia. The diagnosis is further obscured by the lack of clinical and family history and the short period of time for which she was observed.

Several studies have been done measuring the lipid content of samples of blood obtained from pregnant women. Favian and his associates (5) measured fatty acid levels before and after administration of heparin and found that free fatty acids were increased in pregnant women and that these women showed an exaggerated rise in FFA following heparin injection. Measurements of the same women postpartum showed *no significant difference from that of controls. Similarly they also found a decrease in activity of lipoprotein* lipase in the pregnant group.

Quinto and associates (6) in studies of 785 un*complicated pregnancies, found increases in total* lipids, fatty acids, phospholipids, serum ketones, triglycerides and cholesterol. The latter two showed significant elevations only in the third *trimester while the remainder of the values were* elevated throughout pregnancy Mean values were cholesterol 275 mg 100 ml triglycerides 225/100 ml phospholipids 80 mg/100 ml *Post partum measurements showed a return to normal values* by the sixth day

The same group (7) studying patients with toxemia obesity and diabetes, found that toxemic *women manifested an elevation* of triglyceride and phospholipid greater than the non-pregnant or uncomplicated pregnancies. Cholesterol was elevated above normal though lower than the pregnant controls. Mean values were cholesterol 215 mg/100 ml triglycerides 60 mg/100 ml and phospholipids 375 mg/100 ml.

This patient could thus have had elevated lipids secondary to pregnancy complicated by toxemia and indeed some of the changes were no doubt caused by this. However the degree of elevation was much too great to be caused by pregnancy alone and no reports of electrophoretic pattern like the one found have been associated only with pregnancy.

The patient had several characteristics found in those individuals with familial Type I hyperlipoproteinemia—the electrophoretic pattern was Type I with a wide band at the origin representing chylomicrons; the PHLA was extremely low (0.056 mEq/FFA); a good response to injection of *lipoprotein* lipase was obtained (triglycerides fell from 350–145 mg%) lipemia retinalis and creamy supernatant on standing plasma were noted.

Type I electrophoretic patterns have been seen in acute alcoholism, pancreatitis and hypothyroidism. However there is no evidence to support any of these possibilities in this patient. Low PHLA has also been found in hypothyroidism and uncontrolled diabetes but again there is insufficient evidence to support either of these diagnoses. The possibility of lupus is an intriguing one, and was more difficult to dismiss. Glueck and his associates (8) have reported a case with characteristic Type I lipid abnormalities and electrophoretic pattern, and negative lupus prep who was followed and as symptoms and tests for lupus began to appear the lipid abnormalities disappeared. Their patient's PHLA remained low and they were able to demonstrate resistance to heparin in plasma as measured by the patient's thrombin time. They were not, however, able to demonstrate an inhibiting factor to heparin when mixing equal amounts of the patient's plasma with normal post heparin plasma.

Our patient seems remarkably similar in characteristics to that of Glueck. In addition, an inhibitor was found to be present when the patient's plasma was mixed with normal post heparin plasma.

REFERENCES

1. Fredrickson, D. S. & Levy, R. I. Diagnosis and management of hyperlipoproteinemia. Amer J Cardiol 22: 576–583 1968.
2. Fredrickson, D. S. Levy, R. I. & Lees, R. S. Fat transport in lipoproteins. An integrated approach. New Engl J Med 276. 34 94 148 215, 273, 1967
3. Fredrickson, D. S. & Lees, R. S. Familial hyperlipoproteinemia. Chap 22 in The Metabolic Basis of Inherited Disease, Second Edition (ed. J. B. Stanbury J. B. Wyngaarden & D. S. Fredrickson). McGraw-Hill Book Company New York 1966.
4. Fredrickson, D. S. Levy R. I. & Lees, R. S. Fat transport in lipoproteins—an integrated approach. New Engl J Med 276 148–156 1967
5. Pavlan, B., Stork, A. Kacerova, L. et al. Plasma levels of FFA, lipoprotein lipase, and post heparin esterases in pregnancy. Amer J Obstet Gynec *100*. 904–907 1968.
6. Quinto, P. Bottiglioni, F. & Flamigni, C. Metabolic studies in healthy pregnant women. J Obstet Gynaec Brit Comm 74 544–555 1967
7. Quinto, P. Bottiglioni, F. & Flamigni, C. Metabolic studies in icteric, obese and diabetic pregnant women. J Obstet Gynaec Brit Comm 74 556–562, 1967
8. Glueck, C. J. Levy R. I., Glueck, H. I. et al. Acquired type I hyperlipoproteinemia with SLE, dysglobulinemia and heparin resistance. Amer J Med 47 318–324, 1969

Submitted for publication November 29 1972

L. R. Martinez
State University of New York
Downstate Medical Center
450 Clarkson Avenue
Brooklyn, New York 11203
USA

ANNO

The XIV International Congress / Pediatrics will be hel in Buenos Aires, October 3–9 1974

The first day of the Congress is devoted to perinata problems of interest to obstetricians and gynaecologists.

BOOKS R

Fetal Pharmacology Editor Lars Olof Boreus, Elsevier Excerpta Medica, North Holland, Amsterdam 1973 487 pages. Price US$ .3 90.

The complete transactions from the Symposium on Fe tal Pharmacology in Stockholm, September 1971 Drug distribution in pregnancy drugs and autonomic function, drug metabolism and the passage of drugs nd other chemicals into the uterine fluids and their influence on

ANNOUNCEMENTS

The XII I ternational Congress of Pediatrics will be held in Buenos Aires, October 3–9 1974

The first day of the Congress is devoted to perinatal problems of interest to obstetricians and gynaecologists.

For further information write G. International Congress of Pedi. 3177 Buenos Aires, Argentina.

BOOKS RECEIVED

Fetal Pharmacology. Editor Lars Olof Boreus, Elsevier Excerpta Medica, North-Holland, Amsterdam 1973 487 pages. Price US$ .3 90.

The complet transactions from the Symposium on Fetal Pharmacology in Stockholm, September 1971 Drug distribution in pregnancy drugs and autonomic function, drug metabolism and the passage of drugs and other chemicals into the uterine fluids and their influence on the blastocysts devoted to the tions are given the field of f book is w as well as d

ANNOUNCEMENTS

The XIV International Congress of Pediatrics will be held in Buenos Aires, October 3–9 1974

The first day of the Congress is devoted to perinatal problems of interest to obstetricians and gynaecologists.

For further information write General Secretary International Congress of Pediatrics, Casilla de C 3177 Buenos Aires, Argentina.

BOOKS RECEIVED

Fetal Pharmacology Editor Lars Olof Boreus, Elsevier Excerpta Medica, North-Holland, Amsterdam 1973 487 pages. Price US$ 23.90.

The complete transactions from the Symposium on Fetal Pharmacology in Stockholm, September 1971 Drug distribution in pregnancy drugs and autonomic function, drug metabolism and the passage of drugs and other chemicals into the uterine fluids and their influence on the blastocysts are dealt with A special chapte devoted to the future of fetal pharmacology Con tions ar given by the world's most prominent scienti the field of fetal pharmacology and perinatology book is recommended to obstetricians and pediat as well as doctors in gener l practice